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LP1846 LIQUID GUN PROPELLANT DERMAL TOXICITY STUDY IN MALE MINIATURE HANFORD SWINE

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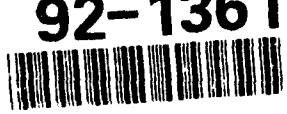

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

LP1846 Liquid Gun Propellant is under development by the U.S. Army as a possible substitute for solid gun propellants. Available toxicity data on liquid gun propellant formulations and/or their major components, indicate toxicities in exposed rats, guinea pigs, rabbits and miniature swine. The major toxic effects of these monopropellants are attributed to the ingredient hydroxylammonium nitrate and result in erythrocyte crenation, Heinz body formation and methemoglobin production.

This range-finding study was conducted to establish a dose response curve, based upon single topical applications of undiluted LP1846 at selected skin surface area exposures in male Hanford Miniature Swine. The data were necessary to select optimal skin surface areas to be exposed in follow-on definitive toxicity studies.

Ten male miniature pigs, 5 per replicate, were randomly assigned, one to each treatment

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19. Abstract (Continued)

(i.e., 15%, 7.5%, 3.8% and 1.9% skin surface area exposed for Replicate A; and 15% 12.5%, 10% and 5% for Replicate B. The negative control animals for each replicate were exposed to water over 15% of their skin surface area. The exposed skin was neither covered, wiped, nor washed once the material was applied.

Moderate to marked increases in methemoglobin levels were observed in all animals receiving LP1846 exposures of at least 5% skin surface area. The numbers of circulating erythrocytes decreased while the numbers of circulating reticulocytes and erythrocytes with Heinz bodies increased. LP1846 was also scored as a severe skin irritant using a 72 hour post-application evaluation scheme. Histologically, treated skin examined 15 days after exposure had epidermal scabs and various degrees of subcutaneous inflammatory cell infiltration. The epithelium, however, was intact. These data suggest that adequate dose response curves can be established in male Hanford Miniature Swine for skin surface exposures to LP1846 of 5% or greater.

GLP: X YES ___NO

Technical Report

LP1846 Liquid Gun Propellant Dermal Toxicity Study in Male Miniature Hanford Swine

FINAL REPORT

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DATE: 11/19/91

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DATE: 4/28/92

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William M. Hitt 11/19/91

Principal Investigator Date

PREFACE

TYPE REPORT: Miniature Swine Dermal Toxicity Study

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SPONSOR:

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STUDY NUMBER: NCTR #6665.01

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CO-PRINCIPAL INVESTIGATORS: Robert M. Parker, Ph.D.
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REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, (Appendix A) and SOPs will be retained in the NCTR Archives

TEST SUBSTANCE: LP1846

INCLUSIVE STUDY DATES: 1 JAN 89 - 30 DEC 90

OBJECTIVE:

The objective for this range-finding study was to establish a dose response curve, based upon single topical applications of neat LP1846 at selected SSAEs in male Hanford Miniature Swine. The data were necessary to select optimal skin surface areas to be exposed in the follow-on definitive toxicity tests in male Hanford Miniature Swine.

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Introduction

LP1846 liquid gun propellant is under development by the U.S. Army as a possible substitute for current solid gun propellants. The limited toxicity data that are available on several liquid gun propellant formulations, as well as their major components, indicate that they are toxic in rats and dogs (1), rabbits, guinea pigs and miniature pigs (2). The major toxic effects involve the RBC by crenation of cells, Heinz body formation and methemoglobin production. The toxic effects with monopropellants such as LP1846 are apparently due to the ingredient hydroxylammonium nitrate (HAN) and produce a nitrate-type of poisoning (Extracted from Freedman, An outline of Liquid Gun Propellants, not dated). The objective for this range-finding study was to establish a dose response curve, based upon single topical applications of neat LP1846 at selected skin surface area exposures (SSAEs) in male Hanford Miniature Swine. The data were necessary to select optimal skin surface areas to be exposed in the follow-on definitive toxicity tests in male Hanford Miniature Swine. Specific aims of the study were to determine the Maximum Tolerated Dose (MTD) of LP1846 within the criteria (an exposure of 15% or less skin surface area) specified in the Sponsor's Scope of Work; to determine appropriate time periods for blood sampling for methemoglobin measurements and the efficacy of selected clinical chemistry assays using pig serum; and to further use the miniature swine to refine SOPs to be used in subsequent definitive toxicity studies.

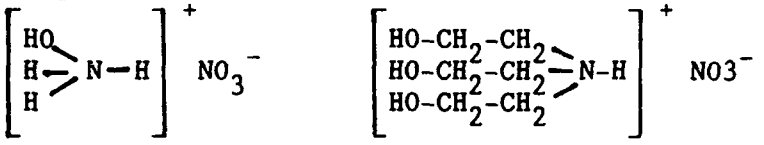
Materials and Methods

LP1846 Procurement and Characterization

The dose solution was LP1846 Liquid Gun Propellant (Lot 1846-03) provided by the U.S. Army Ballistic Research Laboratory, SLCBR-IB-B, Aberdeen Proving Ground, MD 21005-5066. The LP1846 shipment arrived at the NCTR on 05 FEB 90. Characterization and assays of the LP1846 Liquid Gun Propellant was performed by the Sponsor and the Draft of Analytical Procedures (15 June 1989) is attached as Appendix B. No purity analysis was done at the NCTR.

Figure 1. Chemical composition of LP1846

Ingredients (% by weight):
19.2% TEAN (Triethanolammonium nitrate- $C_6H_{16}N_2O_3$)
60.8% HAN (Hydroxylammonium nitrate- $N_2H_4O_4$)
20.0% H_2O



HAN TEAN

Molecular weight: TEAN- 164; HAN- 96
Physical data: Liquid, white to very pale straw colored, odorless
Stability: No decomposition within 48 hours at 70°C
Density (g/cc): 1.42
Viscosity (@0°C): 25 cp
(Extracted from Freedman, An Outline of Liquid Gun Propellants, Not dated)

Control Article

The control article was distilled water.

Determination of Volume per Unit Area

The volume of LP1846 required to cover a known skin surface area was determined by placing a known volume of LP1846 onto a piece of freshly harvested, shaved rat skin and measuring the area covered. Application of 1.0 ml of LP1846 wetted approximately 50 cm^2 of the shaved rat skin (see Appendix C). [Rat skin was used for this purpose because it was readily available from animals humanely euthanatized for other reasons and because excess miniature swine were not available for this determination. Treatment of one of the experimental animals with LP1846 prior to actual testing would have jeopardized that animal's usefulness for the study].

Determination of Surface Area

Skin area exposed for each animal was calculated using the following formula (provided by Sponsor) and multiplying the results by the percent skin area to be wetted:

$$\text{Body Surface Area } (cm^2) = K \times BW^{2/3}$$

Where: K = 9.95 and BW = Body Weight in grams

The swine were weighed and those data, along with the known percent skin surface area to be treated, were used to calculate the volume of agent and the specific area of treatment for each animal. Once the calculations were made the Digiflex Automatic Dispensing Syringe was programmed to dispense the proper amount of agent (see Table 1).

Table 1. Pig Weight and Surface Area; Volume of LP1846

TAG #	KILOGRAMS	TOTAL SURFACE AREA	%REQUIRED	TREATED SURFACE AREA	COMPOUND	VOLUME
REPLICATE A (2/26/90)		SQUARE CENTIMETERS		SQUARE CENTIMETERS		MILLILITERS
109-4	28.30	9240.20	15.00	1386.10	WATER	10.00
118-8	28.40	9261.90	15.00	1389.30	LP1846	27.79
95-2	31.70	9966.20	7.50	747.50	LP1846	14.95
83-6	28.90	9370.30	3.80	356.10	LP1846	7.12
99-10	29.80	9563.90	1.90	181.70	LP1846	3.63
REPLICATE B (3/19/90)						
91-6	33.10	10257.46	15.00	1538.62	WATER	30.77
84-4	34.10	10463.00	15.00	1569.45	LP1846	31.39
106-5	35.30	10707.10	12.50	1338.39	LP1846	26.77
104-6	34.90	10626.10	10.00	1062.61	LP1846	21.25
105-8	35.20	10686.80	5.00	534.34	LP1846	10.69

The amount of water applied to the negative control animal in Replicate A was an arbitrary volume (10 mls) used to cover the designated 15% skin surface area. For Replicate B, the volume of water applied to the negative control animal was that same volume of LP1846 calculated to cover a 15% skin surface area on that same animal.

Animal Maintenance

Young adult male Hanford Miniature Swine were purchased from Charles River Laboratories, Inc. and shipped to NCTR. They were 4-7 months old at delivery and weighed between 21 and 29 kilograms. The animals were uniquely identified by supplier's ear tags. The location for acclimation was Building 14, Rooms 102 and 104. Group pens measured 6' x 18' (housing for 5 animals). The acclimation period was 21 days for Replicate A and 42 days for Replicate B. Physical examinations, base-line hematology/microbiology/clinical chemistry and conditioning to restraint slings were performed during the acclimation period. The animals were transferred to the animal treatment location (Building 14, Room 101) and housed in individual pens (3' x 6') during the time of treatment. The bedding material was pine shavings (Northeastern Products Corporation). Due to Sponsor concern over the possibility of vitamin C being present in the bedding which might interfere with the pharmacodynamics of the induced methemoglobinemia, the bedding was assayed for vitamin C content by the Division of Chemistry, NCTR. Vitamin C was not detected in the pine bedding (see Appendix D). Bedding changes were made daily around water source and twice-weekly for entire run. The temperature was maintained at $23^{o}\pm3^{o}$C with a relative humidity: 50±10%. The light/dark Ratio was 12 hr/12 hr. The animals were fed Purina Commercial Miniature Pig Diet once per day (0.8 - 1.2 Kilogram allotments) and received water ad libitum.

Experimental Design

The range-finding study was divided into 2 replicates of 5 animals each. The animals were randomly assigned, one per each treatment and control. The hair was removed from the treatment area with electric clippers, first with a #10 blade, then with a #40 blade, three days prior to treatment. The animals were weighed and the calculated area of skin over the back and extending down the sides was marked on each animal with indelible ink (see Table 1).

The exposure consisted of a single topical application of neat agent to the skin. The exposed skin was neither covered, wiped, nor washed once the agent was applied. For Replicate A, the skin surface area exposed for the control and high dose was approximately 15%. The upper-middle, low-middle and low doses were 7.5, 3.8 and 1.9% skin surface area (approximately one-half, one-fourth and one-eighth the calculated high dose volume), respectively. SSAEs for Replicate B were determined after review of the Replicate A data and consultation with the Sponsor. Those SSAEs were once again 15% for the control and high dose and 12.5, 10 and 5% for the upper-middle, low-middle and low doses (approximately five-sixths, two-thirds and one-third the calculated high dose volume), respectively (see Appendix A, Protocol Addendum #3). The calculated volume of LP1846 was dispensed via the Digiflex Automated Dispensing Syringe. For Replicate A, the agent was dispensed directly to the skin along the dorsal midline of

each animal and wiped throughout the designated area with a latex gloved hand. With the Sponsor's approval (see Appendix A, Protocol Addendum #5), this procedure was amended for Replicate B where the calculated volume of agent was dispensed into a clean beaker via the Digiflex unit. The agent was then transferred from the beaker to the animal with a 4"x 4" gauze sponge.

All dosing was accomplished with the animals in a restraint sling (Charles River) as described in the literature (3). The animals remained in the sling approximately 4 hours post-exposure at which time they were returned to their individual runs. The animals were returned to the slings only for blood sampling and euthanasia (day 15) for the remainder of the study. Clinical observations were made daily. Skin irritation was graded according to modification of standard published methods (4)(see Figure 2). Body weights were obtained on the day of exposure, study day 8 (body weights for Replicate A were not taken on day 8 - see Appendix E, Deviation 4), and at sacrifice.

Figure 2. Cutaneous Irritation Evaluation

Key to Erythema and Eschar Formation

0 - No erythema
1 - Very slight erythema (barely perceptible)
2 - Well-defined but mild erythema
3 - Moderate to severe erythema
4 - Severe erythema (beet redness) to slight eschar formations (injuries in depth)

Key to Edema Formation

0 - No edema
1 - Very slight edema (barely perceptible)
2 - Slight edema (edges of area well defined by definite raising)
3 - Moderate edema (raised ~1 mm)
4 - Severe edema (raised > 1 mm and extending beyond the area of exposure

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Blood samples for methemoglobin levels were collected via transcutaneous venapuncture from the anterior vena cava on day of allocation, day 1 (0, 2, 4 and 8 hours), and days 2-15. Methemoglobin levels were determined directly from whole blood immediately after sampling using an IL282 Co-Oximeter (Instrumentation Laboratory Inc., Lexington, MA). Blood samples for hematology and clinical chemistry were collected on day of allocation, day 1 (0, 2, 4 and 8 hours), and days 2-5, 8-12 and 15. The hematology evaluations included a complete blood cell count, cell morphology assessment, hemoglobin concentration, hematocrit, mean corpuscular volume, % red cells with Heinz bodies, and, starting 3 days

post-exposure, reticulocyte counts. Clinical chemistry samples were assayed for blood urea nitrogen (BUN), total protein (TP), creatinine (CREA), albumin (ALB) and aspartate aminotransferase (AST) to evaluate liver and kidney function. At the termination of the study (day 15), all animals were weighed and euthanized by iv injection of T-61 Euthanasia Solution (0.3 ml/Kg) in Building 14, Room 101 and then necropsied in Building 14, Room 102. Skin samples (protocol required tissues) were taken from sections of skin exposed to the test and control articles and from an area approximately 5.0 cm away from the edge of the treatment zone. Also, in an effort to determine an ideal sample site, multiple samples were collected from each animal, some more than others, for comparison. See Appendix G for the skin sampling sites for each animal.

Data collection (body weights, clinical observations and day (date) of sacrifice) were entered on the INLIFE automated data collection system. Data acquisition, retrieval, reports and management support were provided by the Division of Research Information and Management Services, NCTR. The Cutaneous Irritation Evaluation records and the methemoglobin records were maintained by the Principal Investigator. All hard copy of study data sheets are stored in the NCTR Archives under the control of the NCTR Quality Assurance Officer. Tissue samples collected during the course of the study were placed in secure storage in the Pathology Division, NCTR.

Results

Methemoglobin Evaluation

The results of Replicates A and B are shown on Table 2 and Figures 3 and 4. In Range-finding Replicate A, only the high dose animal (15% SSAE) had marked (22.7% at 96 hours) and sustained increases in methemoglobin content. In Replicate B, all animals treated with LP1846 had increases in methemoglobin content, although only the high dose animal (15% SSAE), as in Replicate A, had marked (20.3% at 96 hours) and sustained increases. The remaining LP1846 treated animals had moderate increases in methemoglobin content which peaked relatively early (12.0% at 8 hours for the 12.5% SSAE animal; 6.3% at 48 hours for the 10% SSAE animal and 4.5% at 48 hours for the 5% SSAE animal). The methemoglobin level in the Replicate A high dose animal had returned to baseline levels by day 8 while the Replicate B high-dose animal did not return to baseline until day 13.

Clinical Chemistry and Hematology

The results for Replicates A and B are shown on Tables 3A-E and 4A-E. There were no obvious trends in the clinical chemistry data. The hematologic data for all animals dosed at 5% SSAE or greater with LP1846 showed similar trends, although that for the high dose (15% SSAE) animals was more obvious. Following topical application of LP1846 at 5% SSAE or greater, there was a gradual decrease in the numbers of circulating RBCs with a concomitant increase in the percent reticulocytes (Figures 5 and 6). The percent Heinz bodies peaked at levels between 87 and 91%, 48 hours after LP1846 application. These levels steadily decreased over the remainder of the study (Figure 7). Because of unavailability of reagents early in the study, Heinz body counts were performed only for Replicate B.

Table 2. Dermal Toxicity Study of LP1846 Liquid Gun Propellant On Male Miniature Hanford Swine - % Methemoglobin Data

	REPLICATE A					REPLICATE B				
Pig #	118-8	95-2	83-6	99-10	109-4	84-4	106-5	104-6	105-8	91-6
DOSE	15.00%	7.50%	3.80%	1.90%	Control	15.00%	12.50%	10.00%	5.00%	Control
Allocation	1.3	1.3	1.5	1.9	1.4	1.0	1.4	1.0	0.7	0.8
Day 1-0hr	1.2	0.6	1.3	1.6	0.9	0.9	1.2	1.0	1.2	1.0
Day 1-2hr	4.0	1.5	-0.1	1.8	0.8	3.8	1.5	5.9	1.7	0.9
Day 1-4hr	8.0	2.4	1.8	2.3	0.5	4.9	1.8	8.9	1.6	0.9
Day 1-8hr	13.6	3.2	2.0	2.6	2.8	8.9	3.8	12.0	2.9	1.0
Day 2	15.2	2.9	2.3	2.4	1.1	12.4	6.2	7.7	4.4	1.0
Day 3	19.2	2.9	2.3	2.6	1.1	14.6	6.3	5.3	4.5	0.8
Day 4	22.6	2.8	2.0	2.2	1.0	19.7	4.6	4.2	3.2	1.1
Day 5	22.7	2.2	1.9	2.0	1.0	20.3	3.7	3.1	3.2	1.1
Day 6	17.7	2.0	1.6	1.7	1.0	19.9	2.2	2.7	1.4	0.8
Day 7	8.4	1.9	1.2	1.5	0.4	18.0	1.8	1.8	2.3	0.6
Day 8	1.5	1.4	0.2	0.0	1.2	15.5	1.5	1.3	NS	0.9
Day 9	0.4	1.9	1.1	1.7	1.0	11.6	1.0	1.2	1.7	1.1
Day 10	0.5	1.4	2.0	1.4	0.9	6.9	0.8	0.6	1.4	0.8
Day 11	0.4	1.4	1.4	1.6	1.0	5.2	0.5	0.5	1.3	1.0
Day 12	0.3	1.7	1.6	1.4	1.1	2.6	0.6	0.3	NS	0.7
Day 13	0.3	1.7	1.4	1.4	1.0	1.3	0.4	-0.3	0.8	NS
Day 14	0.9	1.6	1.1	1.3	1.2	0.2	0.1	-0.3	0.8	0.8
Day 15	0.5	1.5	1.1	1.5	1.4	-0.2	-0.6	-1.0	0.9	28.0*

NS- No Sample; * Outlier (not included on graph)

Figure 3. Percent Methemoglobin: Replicate A

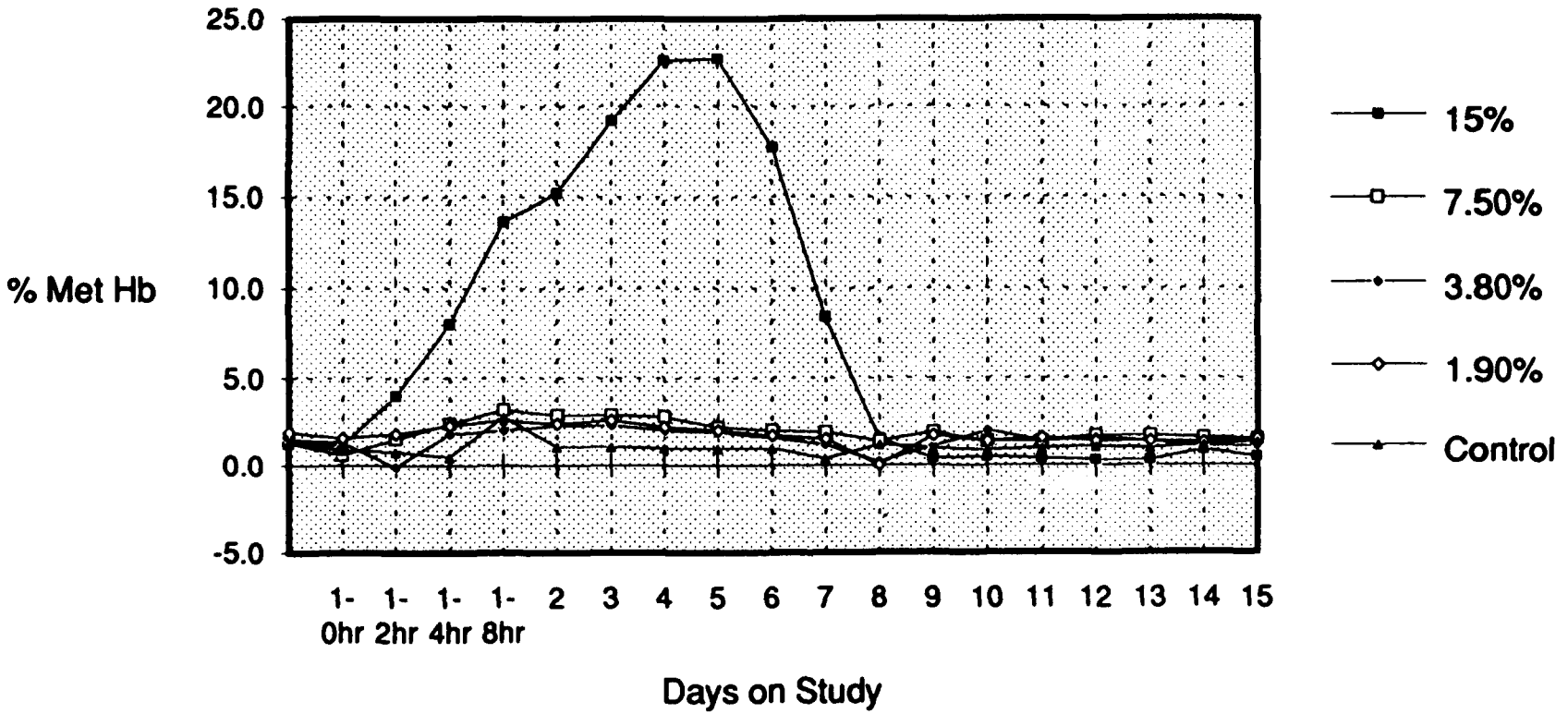


Figure 4. Percent Methemoglobin: Replicate B

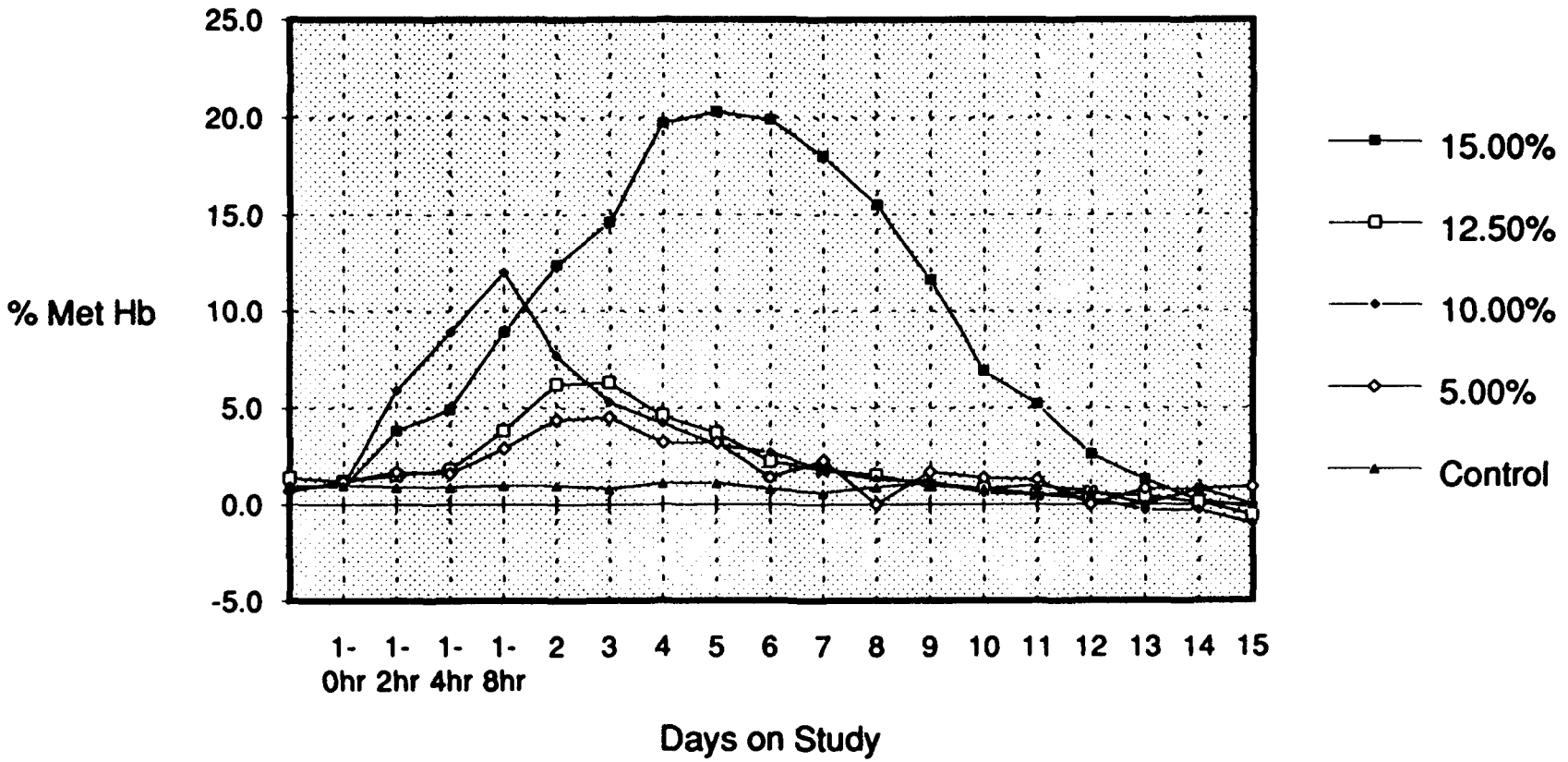


Table 3A. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE A 15% SURFACE AREA CONTROL: PIG [109-4]

DATE	2/21/90	02/26/90-0	2 hr.	4 hr.	8 hr.	2/27/90	2/28/90	3/1/90	3/2/90	3/5/90	3/6/90	3/7/90	3/8/90	3/9/90	3/12/90
BUN mg/dl	10	6	7	8	16	8	7	9	9	10	10	9	7	11	6
T.P. g/dl	6.2	6.6	6.7	7.3	7	7	6.8	6.5	6.3	7.5	7.2	7.3	6.7	7.1	6.6
CREA mg/dl	1.2	0.9	0.9	1.1	1.2	1.2	1.1	1.1	1.1	1	1.1	1.2	1.1	1.2	1.1
ALB g/dl	3.8	4.1	4.2	4.5	4.4	4.2	4.2	4	4.1	4.4	4.3	4.3	4	4.4	4
AST U/L	124	88	37	41	112	39	35	44	31	33	45	88	46	169	284
WBC	*SNA	12	11.9	16.8	20.1	14.3	12.8	13.6	11.4	13.5	15.8	18	13.3	19.8	14.8
RBC	SNA	6.77	7.45	7.62	7.56	7.64	7.43	7.12	7.05	7.65	7.43	7.67	6.56	7.15	6.83
Hgb g/dl	SNA	11.8	12.9	13.2	13.3	13	12.1	12.2	11.9	13.1	13	12.9	11	12	11.3
Hct %	SNA	36.8	40.2	41.5	42	41.9	40	39.4	38.4	41.9	41.3	41.3	35.8	38.7	36.9
MCV fL	SNA	55	55	55	56	55	54	55	55	55	56	54	55	54	54
MCH pg	SNA	17.7	17.6	17.6	17.9	17.1	16.3	17.4	17	17.2	17.7	17	16.9	16.9	16.7
MCHC g/dl	SNA	31.8	31.9	31.7	31.5	30.8	30.1	30.9	30.9	30.9	31.4	31.1	30.6	30.9	30.5
SEG %	SNA	44	51	57	40	48	33	41	41	44	38	45	38	46	42
EOS %	SNA	2	1	0	0	3	2	2	1	0	1	2	0	0	1
LYM %	SNA	50	43	42	54	44	60	53	56	54	59	49	59	53	52
MON %	SNA	4	4	1	6	5	5	4	2	2	2	4	3	1	5
RETIC %RBC	-----	-----	-----	-----	-----	-----	1.4	1.8	1.1	1.1	0.9	0.9	0.8	0.9	0.7
RBC/100WBC	SNA	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Sample Not Acceptable

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobim; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Table 3B. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE A 15% SURFACE AREA TREATED WITH LP1846: PIG [118-8]

DATE	2/21/90	02/26/90-0	2 hr.	4 hr.	8 hr.	2/27/90	2/28/90	3/1/90	3/2/90	3/5/90	3/6/90	3/7/90	3/8/90	3/9/90	3/12/90
BUN mg/dl	10	6	8	8	10	11	7	10	12	9	8	6	8	6	6
T.P. g/dl	6.8	6.5	6.5	7.1	6.7	6.1	6.3	7.2	7.6	7.1	7.5	7.1	6.7	6.9	6.6
CREA mg/dl	1.2	1	1	1.2	1.4	1.3	1.1	0.9	1.1	1	0.9	1.2	1.1	1.2	1
ALB g/dl	4.2	4.1	4	4.6	4.1	3.8	3.7	3.9	3.9	3.8	4	3.8	3.6	3.7	3.5
AST U/L	83	108	35	37	41	46	38	31	34	36	30	42	60	51	36
WBC	13.9	15.2	12	10.5	12.8	14.3	32.3	39.2	33.9	22	12.7	12.7	10.1	22.9	12.3
RBC	7.96	6.79	6.58	7.8	7.1	6.98	6.49	6.56	6.11	3.54	3.19	3.77	4	3.7	4.36
Hgb g/dl	13.8	12.3	11.7	14.2	13	14.1	13.9	14.7	13.9	8.9	8.2	9.5	9.8	8.9	10.2
Hct %	44.8	38.8	37.4	44.7	41.5	39.8	36.8	38.2	36.7	30.3	28.2	32.5	34.1	30.3	34.5
MCV fL	57	58	57	58	59	57	57	58	60	85	87	85	85	81	78
MCH pg	17.5	18.3	18.1	18.5	18.5	20.1	21.4	22.5	22.7	25	25.4	25.1	24.4	23.9	23.3
MCHC g/dl	30.8	31.5	31.3	31.7	31.1	35	37.7	38.4	37.6	29.3	28.7	29.1	28.6	29.3	29.5
SEG %	26	41	44	61	52	52	27	32	29	30	32	23	23	23	28
EOS %	1	1	0	0	1	0	1	1	1	2	0	2	2	0	0
LYM %	70	55	53	36	43	43	66	63	69	65	62	72	73	77	71
MON %	3	3	3	3	4	5	6	4	1	3	6	3	2	0	1
RETIC %RBC	-----	-----	-----	-----	-----	-----	3.5	2.4	17.9	21.3	21.8	13.4	9.2	4.4	2.3
RBC/100WBC	0	0	0	0	0	6	3	5	25	14	26	9	7	5	3

* Sample Not Acceptable

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobim; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Table 3C. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE A 7.5% SURFACE AREA TREATED WITH LP1846: PIG [95-2]

DATE	2/21/90	02/26/90-0	2 hr.	4 hr.	8 hr.	2/27/90	2/28/90	3/1/90	3/2/90	3/5/90	3/6/90	3/7/90	3/8/90	3/9/90	3/12/90
BUN mg/dl	9	7	6	8	15	9	9	10	10	6	10	10	8	9	6
T.P. g/dl	6.4	6.8	6.8	7.3	6.4	6.4	6	6.9	7.3	6.1	7.4	7.4	7.1	6.4	6.6
CREA mg/dl	1.1	1	1.2	1.1	1.2	1	1	1	0.6	0.8	1.1	1.2	1.1	1.2	1.2
ALB g/dl	3.9	4.3	4.3	4.7	4.1	3.9	3.4	3.6	3.7	QNS*	3.8	3.9	3.8	3.3	3.5
AST U/L	176	31	44	65	462	45	50	94	393	QNS	143	39	41	214	31
WBC	21.2	SNA**	15.8	22.3	21.9	15.3	13.8	13.4	12	SNA	14.2	15.2	17.6	12.4	12.8
RBC	7.03	SNA	7.68	7.6	7.31	7.16	6.8	6.45	6.02	SNA	6.82	7.28	6.91	5.52	5.93
Hgb g/dl	12.4	SNA	14	14.1	13.3	13.1	12.8	12	12.2	SNA	13	13.9	12.9	12.5	11.2
Hct %	40.5	SNA	44.8	45	41.8	42.8	40.2	37.4	35.4	SNA	41	44.1	41.6	33	35.6
MCV fL	58	SNA	59	60	58	60	59	58	59	SNA	60	61	60	60	60
MCH pg	17.7	SNA	18.6	18.8	18.5	18.3	18.9	18.7	20.3	SNA	19.1	19.2	18.7	22.5	18.9
MCHC g/dl	30.5	SNA	31.1	31.1	31.7	30.3	31.8	32	34.3	SNA	31.6	31.5	30.8	37.5	31.4
SEG %	37	SNA	49	57	55	47	SNA	40	41	SNA	44	40	52	41	31
EOS %	1	SNA	1	1	1	0	SNA	3	2	SNA	1	5	1	1	1
LYM %	57	SNA	49	39	41	47	SNA	52	56	SNA	50	49	42	56	66
MON %	5	SNA	1	3	3	6	SNA	5	1	SNA	5	6	5	2	2
RETIC %RBC	-----	-----	-----	-----	-----	-----	SNA	2.1	2	SNA	2.1	2.8	2.2	2.4	0.9
RBC /100WBC	0	SNA	0	0	0	0	SNA	0	0	SNA	0	0	0	0	0

* Quantity Not Sufficient; ** Sample Not Acceptable

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobim; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Table 3D. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE A 3.8% SURFACE AREA TREATED WITH LP1846: PIG [83-6]

DATE	2/21/90	02/26/90-0h	2 hr.	4 hr.	8 hr.	2/27/90	2/28/90	3/1/90	3/2/90	3/5/90	3/6/90	3/7/90	3/8/90	3/9/90	3/12/90
BUN mg/dl	11	8	8	9	16	10	8	11	11	9	8	8	8	7	4
T.P. g/dl	6.7	6.5	6.4	6.8	6.7	6.5	6.4	6.5	6.7	6.2	6.8	6.8	6.6	6.5	6.7
CREA mg/dl	1.1	1.1	1.2	1.2	1.1	1.1	1.1	0.9	1.2	0.9	1.2	1.3	1.3	1.1	1.1
ALB g/dl	4.1	4.1	4	4.3	4.2	4.1	3.8	3.7	3.8	4	3.9	3.9	3.7	3.7	3.9
AST U/L	84	31	34	40	38	49	33	434	QNS*	258	46	45	28	66	38
WBC	11.8	7.3	11	18.3	17.1	12.8	13	9.5	12	11.1	13.2	9.4	12.1	12.3	11.1
RBC	7.14	5.19	6.1	6.93	6.46	6.51	6.4	5.95	6.13	5.66	6.07	6.45	5.84	5.93	6.17
Hgb g/dl	13.8	10.8	12.3	14.2	13	13	12.9	13.2	13.4	13.4	12.8	13.5	12.3	12.6	12.9
Hct %	45.1	33.4	38.9	44.6	40.2	41.3	41.7	38.4	40	37.7	41.3	43.8	39.8	39.8	40.3
MCV fL	64	64	64	65	63	63	65	64	65	66	68	68	68	67	65
MCH pg	19.4	21.1	20.4	20.7	20.4	19.9	20.1	22.1	21.9	23.6	21.1	21	21	21.3	20.9
MCHC g/dl	30.5	32.2	31.6	31.6	32.1	31.1	30.9	34	33.3	35.2	30.8	30.7	30.7	31.5	31.8
SEG %	40	38	SNA**	59	50	51	46	39	39	43	41	38	50	40	36
EOS %	3	0	SNA	2	1	0	2	2	0	2	1	1	2	2	1
LYM %	52	58	SNA	38	49	47	46	56	56	53	54	59	47	55	58
MON %	4	4	SNA	1	0	2	6	3	5	2	4	2	1	3	5
RETIC %RBC	-----	-----	-----	-----	-----	-----	1.8	1.6	1.9	2.5	2	2.8	4.6	3.1	2.6
RBC /100WBC	0	0	SNA	0	0	0	0	0	0	0	0	0	0	0	0

* Quantity Not Sufficient; ** Sample Not Acceptable

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobim; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Table 3E. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE A 1.9% SURFACE AREA TREATED WITH LP1846: PIG [99-10]

DATE	2/21/90	02/26/90-0	2 hr.	4 hr.	8 hr.	2/27/90	2/28/90	3/1/90	3/2/90	3/5/90	3/6/90	3/7/90	3/8/90	3/9/90	3/12/90
BUN mg/dl	17	6	7	7	13	8	7	7	10	6	7	QNS*	7	6	6
T.P. g/dl	6.7	6.4	6.2	7.3	6.6	6.4	6	6.4	7.2	6.6	7.1	7.1	6.7	6.5	6.3
CREA mg/dl	1.2	0.6	0.9	1.3	1	1	0.9	0.8	1	1.1	1.1	1.2	1.2	1.1	1.1
ALB g/dl	4	4.3	4.3	4.7	4.2	4	3.6	3.5	3.9	3.8	3.9	4.1	4	3.9	3.9
AST U/L	35	35	34	47	41	34	29	26	45	41	39	104	251	112	29
WBC	15	SNA**	11.5	13.3	17.7	20.3	17.3	14.4	17.8	16.6	17.7	SNA	15.5	14.6	14.1
RBC	6.81	SNA	5.82	7.25	5.96	6.41	5.25	5.26	5.85	6	6.35	SNA	6.16	6.52	5.67
Hgb g/dl	12.7	SNA	11.3	14.4	11.6	12.5	10.5	10.6	11.7	12.2	12.8	SNA	12.2	13	11.4
Hct %	41.9	SNA	35.8	45.1	37.2	40.5	33.1	33.6	37.5	38.8	40.9	SNA	39.3	41.4	36.3
MCV fL	62	SNA	62	63	63	63	63	64	64	64	64	SNA	64	64	64
MCH pg	18.8	SNA	19.7	20.1	19.7	19.4	20	20.2	20.1	20.3	20.2	SNA	19.8	20	20.1
MCHC g/dl	30.4	SNA	31.5	31.8	31.1	30.5	31.6	31.4	31	31.2	31.3	SNA	30.8	31.3	31.2
SEG %	31	SNA	46	48	50	57	51	52	31	32	41	SNA	42	32	44
EOS %	1	SNA	0	0	1	0	0	3	1	0	2	SNA	2	2	3
LYM %	65	SNA	52	49	47	38	47	42	63	65	54	SNA	51	61	51
MON %	3	SNA	2	3	2	5	2	3	5	3	3	SNA	5	5	2
RETIC %RBC	-----	-----	-----	-----	-----	-----	2	1.8	2.1	1.9	2	SNA	1.5	1.2	0.8
RBC /100WBC	0	SNA	0	0	0	0	0	0	0	0	0	SNA	0	0	0

* Quantity Not Sufficient; ** Sample Not Acceptable

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count;

Table 4A. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE B 15% SURFACE AREA CONTROL: PIG [91-6]

DATE	3/15/90	3/19/90-0hr	2 hr.	4 hr.	8 hr.	3/20/90	3/21/90	3/22/90	3/23/90	3/26/90	3/27/90	3/28/90	3/29/90	3/30/90	4/2/90
BUN mg/dl	11	10	12	12	12	9	11	11	14	10	11	12	11	10	8
T.P. g/dl	7.3	7.2	7.4	7	7.2	7.5	7.2	7.3	7	6.9	6.7	7.1	7.3	7.2	7
CREA mg/dl	1.1	1.3	1.1	1	1.1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.9	1.1
ALB g/dl	4.3	4.2	4.1	4.2	4.1	4.4	4.3	4.3	4.1	4	3.9	4.2	4.4	4.2	4.2
AST U/L	44	98	42	43	93	40	27	29	101	34	34	38	34	24	46
WBC	15.2	14	15	21	20.9	13.6	16.9	16.4	14.8	15.5	15.4	15.6	15.4	14.2	12.6
RBC	6.78	6.92	6.78	6.47	6.59	6.77	5.84	6.57	6.47	6.09	5.83	5.81	6.24	5.94	5.83
Hgb g/dl	13.7	13.8	13.5	12.8	12.4	13.4	11.9	13	13.2	12.4	12	12	12.5	12.2	12
Hct %	44.4	44.4	42.9	42.1	43.6	43.8	38.5	43	42.9	40.2	38.6	38.3	41.1	39.4	38.3
MCV fL	65	64	63	65	66	65	66	65	66	66	66	65	65	66	66
MCH pg	20.2	20	20	19.7	18.8	19.7	20.3	19.7	20.4	20.5	20.6	20.6	20	20.5	20.6
MCHC g/dl	30.8	31	31.4	30.3	28.3	30.3	30.6	30.1	30.8	31	31	31.3	30.3	30.9	31.1
SEG %	44	37	57	65	SNA*	39	42	53	39	38	43	48	41	48	45
EOS %	0	4	2	0	SNA	3	1	2	1	3	1	1	2	2	3
LYM %	56	59	41	35	SNA	58	56	42	56	58	56	51	57	49	52
MON %	0	0	0	0	SNA	0	1	3	4	1	0	0	0	1	0
RETIC %RBC	------	------	------	------	------	------	0.9	1.1	1.5	0.9	1	0.8	0.9	0.7	0.5
RBC /100WBC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heinz Bodies**	------	------	------	------	------	------	0	0	0	0	0	0	0	0	0

* Sample Not Acceptable; **% RBC with Heinz Bodies

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobin; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Table 4B. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE B 15% SURFACE AREA TREATED WITH LP1846: PIG [84-4]

DATE	3/15/90	3/19/90-0h	2 hr.	4 hr.	8 hr.	3/20/90	3/21/90	3/22/90	3/23/90	3/26/90	3/27/90	3/28/90	3/29/90	3/30/90	4/2/90
BUN mg/dl	11	6	8	9	10	8	6	5	5	5	5	6	6	8	6
T.P. g/dl	6.9	7	7	6.9	6.5	6	5.3	6.2	6.6	6.9	6.7	7	7.1	7.1	6.2
CREA mg/dl	1.2	1.1	1.1	1	1	0.9	1	0.8	0.7	0.7	0.7	0.7	0.9	0.8	1
ALB g/dl	4.4	4.2	4.2	4.3	4	3.5	3.1	3.3	3.5	3.5	3.4	3.7	3.6	3.7	3.6
AST U/L	QNS*	40	39	44	55	36	23	28	22	29	31	193	40	39	57
WBC	9.8	12.5	15.1	18.8	SNA**	15.3	11	15.1	22.9	24.5	17.9	13.1	10.3	8.9	8.6
RBC	6.91	6.76	6.45	6.48	SNA	6.26	5.22	4.76	5.33	4.93	4.14	3.97	4.22	4.29	3.65
Hgb g/dl	13.8	13.6	12.8	13	SNA	12.8	11.8	11.5	13.3	13	10.9	11.4	11.4	11.8	9.8
Hct %	44.3	42.2	41.1	41.8	SNA	40.5	34.1	31.2	34.9	37	32	32.6	35.6	37.2	31.3
MCV fL	64	62	64	64	SNA	65	65	65	65	74	76	81	83	86	85
MCH pg	19.9	20.2	19.8	20.1	SNA	20.4	22.5	24	24.7	26.4	26.1	28.4	26.7	27.3	26.7
MCHC g/dl	31	32.2	30.9	31	SNA	31.3	34.3	36.7	37.9	35.3	34	34.7	31.9	31.6	31.1
SEG %	32	38	52	66	SNA	71	54	61	54	46	43	41	33	28	17
EOS %	2	4	0	0	SNA	0	2	1	1	4	3	1	3	1	2
LYM %	66	53	47	33	SNA	28	41	36	43	48	54	56	63	69	80
MON %	0	5	1	1	SNA	1	3	2	2	2	0	2	1	2	1
RETIC %RBC	------	------	------	------	------	------	1.3	2.6	5.8	10.8	11.5	12.1	8.7	7.2	3.6
RBC /100WBC	0	0	0	0	0	0	0	1	3	2	1	0	0	0	0
Heinz Bodies***	------	------	------	------	------	------	91.3	90.6	84.7	76.9	48.6	37.6	22.4	15.2	5.7

* Quantity not sufficient; **Sample Not Acceptable; *** % RBC with Heinz Bodies

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobim; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Table 4C. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE B 12.5% SURFACE AREA TREATED WITH LP1846: PIG [106-5]

DATE	3/15/90	3/19/90-0	2 hr.	4 hr.	6 hr.	3/20/90	3/21/90	3/22/90	3/23/90	3/26/90	3/27/90	3/28/90	3/29/90	3/30/90	4/2/90
BUN mg/dl	9	6	10	11	10	10	13	5	7	7	8	7	6	9	8
T.P. g/dl	7.1	7.2	7.2	7	6.9	6.4	6	6.8	7.6	6.8	6.8	7.4	7.1	7.4	7
CREA mg/dl	1.4	1.4	1.3	1.3	1.3	1.4	1.3	1.1	1.1	1	1.1	1.2	1.2	1.5	1.1
ALB g/dl	4.6	4.5	4.7	4.6	4.3	4.3	3.8	3.8	4	3.8	3.9	4.2	4.2	4.4	4.2
AST U/L	46	39	64	50	57	39	32	33	35	32	44	45	36	139	35
WBC	10.9	7.3	18.9	16.2	12.5	15	9.6	11.5	14.5	14.6	15.5	11.6	12.3	12.4	9.9
RBC	7.16	7.34	6.9	6.46	6.63	6.73	6.01	5.46	6.31	5.71	5.68	6.49	5.86	6.51	5.34
Hgb g/dl	13.8	13.9	12.7	13.1	13	12.7	12.4	11.5	13.9	12.8	12.3	14.2	12.9	14.4	12
Hct %	42.6	44	41.7	40.2	40.4	40.9	36.5	33.6	39	35.9	35.8	41.1	37	41.4	34.6
MCV fL	59	60	61	62	61	61	60	61	61	63	63	63	63	63	64
MCH pg	19.2	19	18.5	20.4	19.6	18.8	20.5	21	21.9	22.5	21.6	21.8	21.8	22	22.3
MCHC g/dl	32.2	31.5	30.4	32.6	32	30.8	33.6	34	35.5	35.6	34.3	34.5	34.6	34.5	34.3
SEG %	46	22	31	29	41	71	21	24	27	26	27	32	24	21	18
EOS %	1	4	0	0	4	0	6	2	3	0	1	1	3	1	2
LYM %	51	74	69	69	55	26	68	74	69	74	72	67	73	78	79
MON %	2	0	0	2	0	3	5	0	1	0	0	0	0	0	1
RETIC %RBC	------	------	------	------	------	------	2.8	2.9	1.9	1.4	1.6	1.1	0.9	0.7	0.5
RBC /100WBC	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Heinz Bodies*	------	------	------	------	------	------	90.7	84.7	80.5	69.3	54.6	51.3	52.7	37.8	36.9

* % RBC with Heinz Bodies

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobim; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Table 4D. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE B 10% SURFACE AREA TREATED WITH LP1846: PIG [104-6]

DATE	3/15/90	3/19/90-0hr	2 hr.	4 hr.	6 hr.	3/20/90	3/21/90	3/22/90	3/23/90	3/26/90	3/27/90	3/28/90	3/29/90	3/30/90	4/2/90
BUN mg/dl	10	10	10	12	19	12	8	9	9	9	9	8	8	8	7
T.P. g/dl	6.6	7.2	7	7	6.6	6.4	6.1	6.5	6.8	6.9	6.8	7.4	7.1	7.2	6.9
CREA mg/dl	1.1	1.6	1.6	1.6	1.4	1.4	1.2	1.2	1.2	1.4	1.4	1.4	1.3	1.4	1.3
ALB g/dl	4.4	4.4	4.2	4.4	4	3.6	3.6	3.5	3.7	3.7	3.7	4.1	3.8	4	4
AST U/L	234	87	65	97	95	64	41	35	43	44	39	38	36	41	36
WBC	17.3	16.3	17.7	26.8	22.9	13	13.8	12.9	12.1	12.3	11.7	11.4	11.7	11.9	10.2
RBC	6.25	7.58	6.88	6.88	6.56	7.19	5.69	6.01	5.79	5.87	5.61	6.08	5.49	5.44	5.62
Hgb g/dl	11.8	14.2	13	13.4	12.4	13.9	11.8	12.6	12.5	13.2	12.1	13.4	12.2	12.3	12.5
Hct %	37.5	45.4	41.6	41.6	40	42.8	34.9	36.9	35.6	37.9	36.7	40.1	36.5	36.3	37.9
MCV fL	60	60	60	61	61	60	61	61	61	64	65	66	66	66	67
MCH pg	18.8	18.8	18.9	19.5	19	19.3	20.7	20.9	21.5	22.6	21.6	21.9	22.1	22.5	22.2
MCHC g/dl	31.3	31.1	31.1	32.1	30.9	32.1	33.6	34	35	34.9	33	33.1	33.3	33.8	32.7
SEG %	31	26	62	59	71	58	41	55	52	42	47	39	43	45	37
EOS %	0	1	0	1	1	1	2	1	2	2	0	2	3	3	4
LYM %	69	72	36	38	27	40	54	42	46	56	53	59	54	52	58
MON %	0	1	2	2	1	1	3	2	0	0	0	0	0	0	1
RETIC %RBC	------	------	------	------	------	------	1.6	2.4	2.6	2.9	2.5	2.7	1.7	1.3	1.2
RBC /100WBC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heinz Bodies*	------	------	------	------	------	------	87.4	85.2	81.6	72.2	58.3	54.1	46.3	41.8	33.2

* % RBC with Heinz Bodies

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobim; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Table 4E. CLINICAL CHEMISTRY AND HEMATOLOGY DATA FOR REPLICATE B 5% SURFACE AREA TREATED WITH LP1846: PIG [105-8]

DATE	3/15/90	3/19/90-0hr	2 hr.	4 hr.	8 hr.	3/20/90	3/21/90	3/22/90	3/23/90	3/26/90	3/27/90	3/28/90	3/29/90	3/30/90	4/2/90
BUN mg/dl	9	7	12	9	------	9	11	10	18	------	6	7	8	6	7
T.P. g/dl	7.2	6.8	7.1	6.9	------	6.8	6.3	7.2	7.4	------	6.8	6.9	7.1	7.5	7.5
CREA mg/dl	1.2	1.3	QNS*	1.2	------	1.2	1	1.2	1.1	------	1	0.8	1.2	1.1	1.1
ALB g/dl	4.7	4.4	4	4.4	------	4.2	3.8	3.8	4	------	4	4.1	4.1	4.1	4.4
AST U/L	86	144	210	46	------	101	33	39	36	------	37	115	196	32	63
WBC	12.1	17.2	22.1	23.5	20.4	14.5	17.3	15.4	15.7	------	13.7	14.3	11.4	------	10
RBC	7.54	7.22	7.3	6.58	5.08	5.71	5.98	6.5	6.8	------	6.51	6.46	6.92	------	5.76
Hgb g/dl	13.9	13.4	13.5	13.6	11.1	10.5	11.6	13	14	------	13.7	13.7	14.7	------	12.9
Hct %	44.5	42.8	42.6	39.5	30.3	33.9	35.8	39.2	41.6	------	42.6	42.2	45.7	------	39.2
MCV fL	59	59	58	60	59	59	60	60	61	------	65	65	66	------	68
MCH pg	18.4	18.6	18.7	20.7	21.8	18.4	19.3	20.1	20.6	------	21	21.2	21.2	------	22.4
MCHC g/dl	31.1	31.2	31.7	34.4	36.5	30.7	32	33	33.6	------	32.1	32.4	32.1	------	32.8
SEG %	38	39	59	66	71	60	52	49	41	------	37	41	37	------	31
EOS %	2	3	0	0	0	1	4	2	3	------	2	1	3	------	3
LYM %	58	57	40	34	29	37	39	48	55	------	61	57	58	------	66
MON %	2	1	1	0	0	2	5	1	1	------	0	1	2	------	0
RETIC %RBC	------	------	------	------	------	------	1.3	3.6	4.5	------	3.6	2.9	3.1	------	1.9
RBC /100WBC	0	0	0	0	0	0	0	1	1	------	0	0	0	------	0
Heinz Bodies**	------	------	------	------	------	------	88.9	72.3	71.3	------	54.9	50.3	42.7	------	32.4

* Quantity not sufficient; ** % RBC with Heinz Bodies

BUN - Blood Urea Nirogen; T.P. - Total Protein; CREA - Creatinine; ALB - Albumin; AST - Aspartate aminotransferase; WBC - White blood cell count; RBC - Red blood cell count; Hgb - Hemaglobim; Hct - Hematocrit; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemaglobin: MCHC - Mean Corpuscular Hemaglobin Concentration; SEG - Segmented Neutrophiles; EOS - Eosinphiles; LYM - Lymphocytes; MON - Monocytes RETIC - Reticulocytes

Figure 5. Comparison of RBC Counts in High Dose and Control Animals

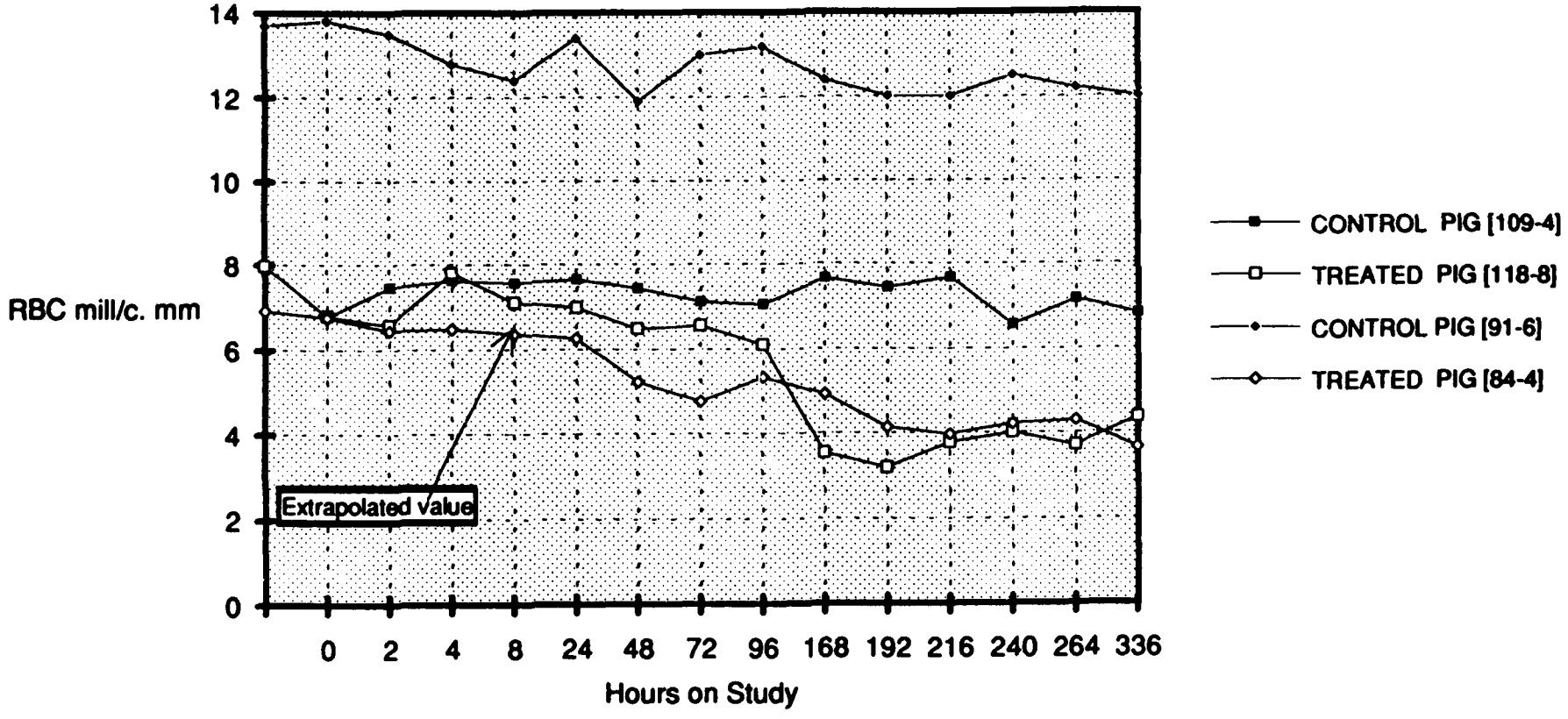


Figure 6. Comparison of Reticulocyte Count in High Dose and Control Animals

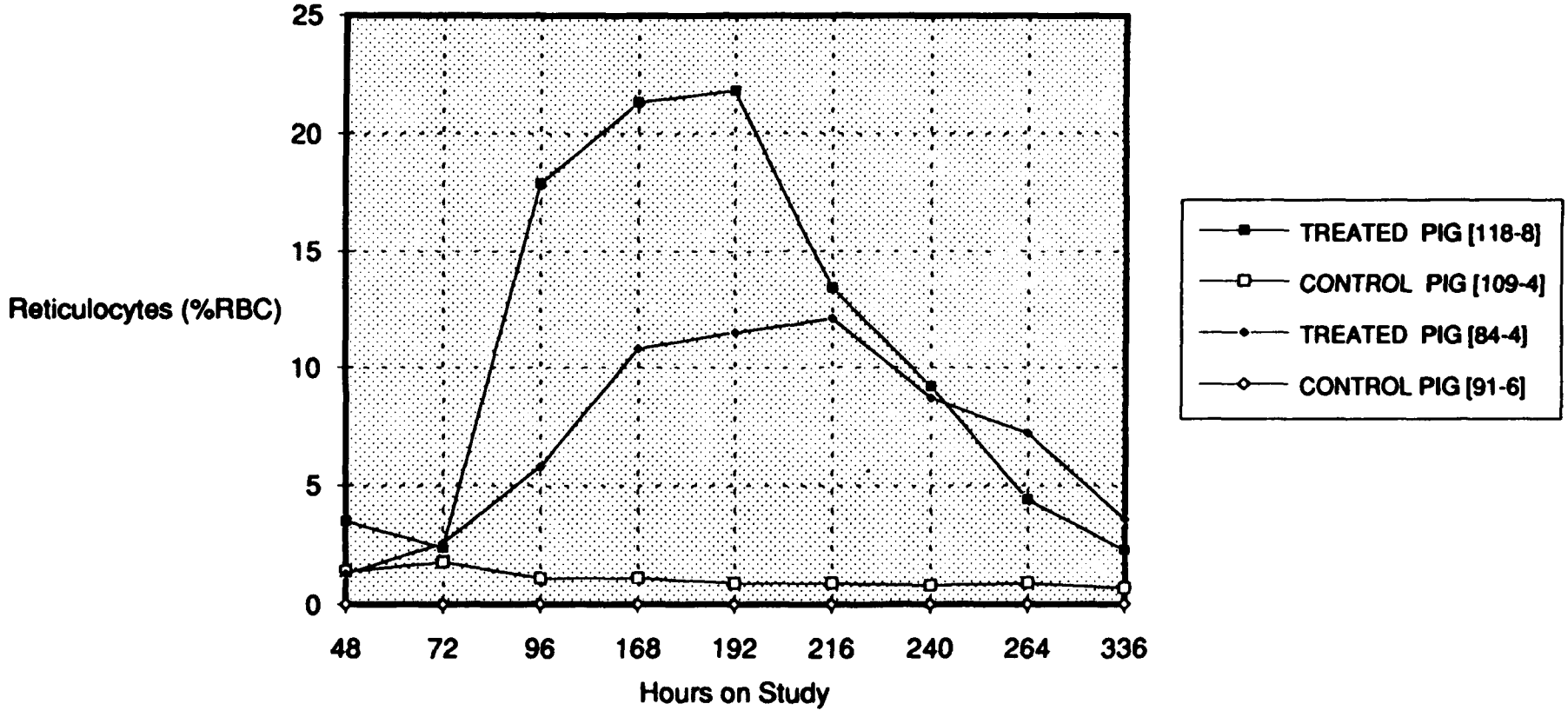
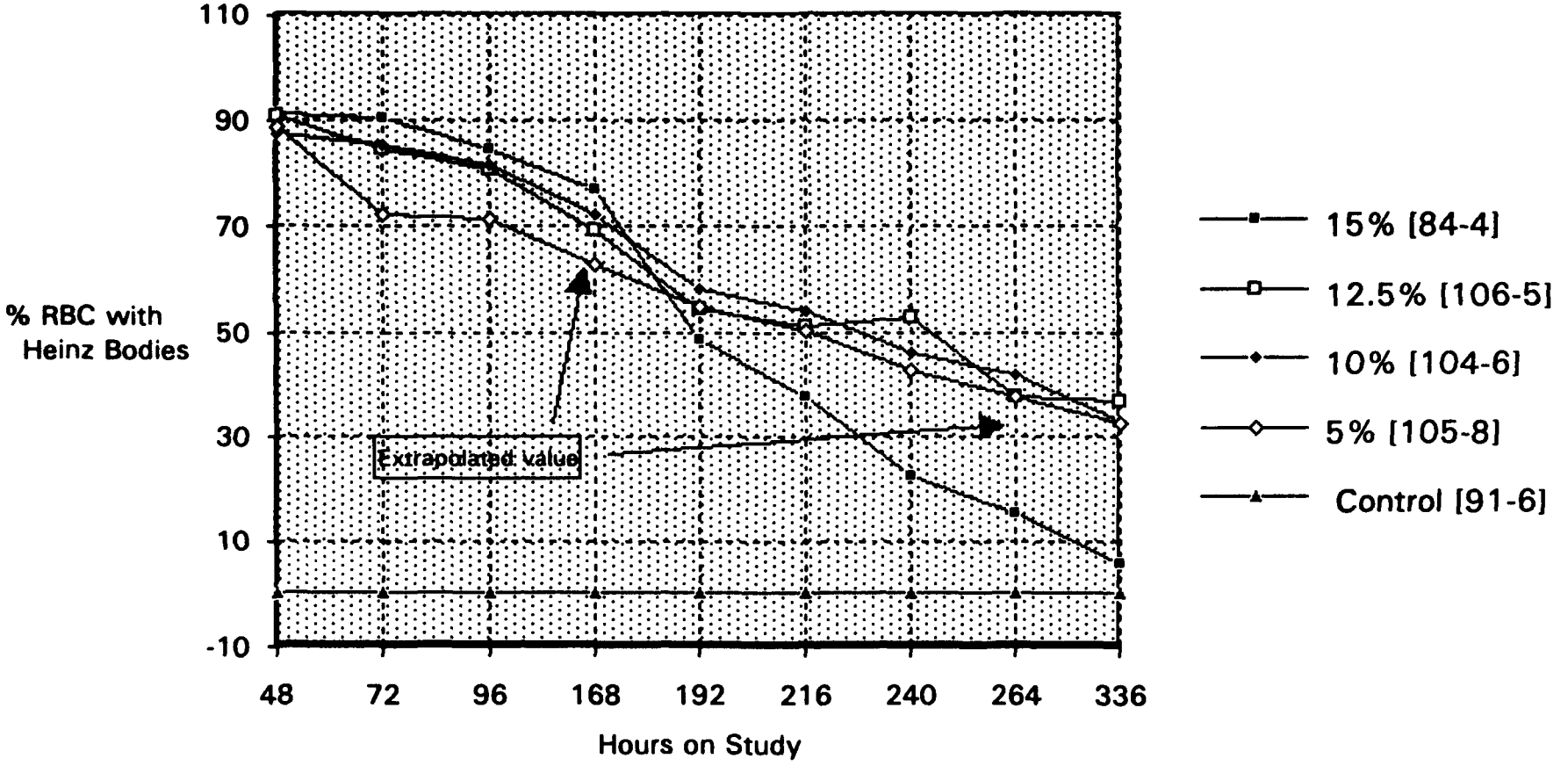


Figure 7. Percent RBC with Heinz Bodies: Replicate B



Cutaneous Irritation Evaluation

All evaluations of cutaneous irritation were made with the animal resting in a restraint sling. The evaluations were made and recorded for each animal at 4, 24, 48, and 72 hours after treatment. The treated skin was scored for erythema and edema (Table 5) using the numerical scale in Figure 2. The sums of each reading (score for the erythema and score for edema added together for each time period) were subtotaled after the 72 hour reading and divided by 4 (the number of evaluation periods) to provide a mean score termed the Primary Cutaneous Irritation Index (PCI). The irritancy of the test article was defined as:

a) Non-irritant..... PCI < 0.5
b) Slightly Irritant..... 0.5 $\leq$ PCI < 3.0
c) Moderately Irritant... 3.0 $\leq$ PCI < 5.0
d) Severely Irritant..... 5.0 $\leq$ PCI < 8.0

The cutaneous irritancy score for each animal was expected to be similar because each animal received a topical application of undiluted agent with only the total skin surface exposed being varied. As expected, there was little PCI variation within the two replicates. However, a slight difference in PCI was recorded between the two replicates although in both replicates, the agent was scored as severely irritant. The difference in scoring occurred in the scores for edema in Replicate B which were judged more severe earlier than those for Replicate A.

Application of the agent appeared to cause some degree of immediate discomfort, exhibited by the animal's movement in the restraint sling and by increased "grunting." After approximately five (5) minutes, the treated area of skin became pink and progressed to severe erythema by 48 hours after agent application. Vesicle formation occurred in the 24 to 48 hour period. Vesicles progressed to pustules during the 24 to 48 hour period and surface crusting occurred as pustules ruptured. Healing progressed through the remainder of the 15 day observation period.

Histopathology

Histopathology was performed on sections of skin exposed to the test and control articles. Additional skin tissue samples were collected from sites no less than 5.0 cm away from the edge of the treatment area. Because agent was applied to skin on both sides of the dorsal midline, skin specimens were taken from both sides of the midline for purposes of comparison. Additional samples were taken from the control and high dose animals to correspond with each of those taken from the remaining pigs (Appendix G).

Representative lesions from LP1846 treated skin varied from that characterized by a few, widely distributed inflammatory cells (Grade 1) to those with marked inflammatory cell infiltrates, focal epidermal necrosis, subepidermal and occasional periadnexal edema (Grade 5). A superficial layer of serum exudate (crust) was often present on the epidermis of sections graded 3-5. The epithelium was intact in all skin sections examined. The score for each individual skin sample is found in Appendix H. No gross lesions other than exposed skin were observed at necropsy.

Table 5. Cutaneous Irritation Scoring

REPLICATE A

		OBSERVATION PERIOD									
		4 Hours		24 Hours		48 Hours		72 Hours			
Animal ID	Treatment	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Total	PCI
118-8	15%SSA * LP1846	2	0	3	3	4	3	4	3	22	5.50
95-2	7.5%SSA LP1846	1	0	3	3	4	3	4	3	21	5.25
83-6	3.8%SSA LP1846	2	0	3	3	4	3	4	3	22	5.50
99-10	1.9%SSA LP1846	2	0	3	3	3	4	4	4	23	5.75
109-4	15%SSA Water	0	0	0	0	0	0	0	0	0	0

REPLICATE B

		OBSERVATION PERIOD									
		4 Hours		24 Hours		48 Hours		72 Hours			
Animal ID	Treatment	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Total	PCI
84-4	15%SSA LP1846	1	1	3	3	4	4	4	4	24	6.00
106-5	12.5%SSA LP1846	1	1	3	3	4	4	4	4	24	6.00
104-6	10%SSA LP1846	2	1	3	3	4	4	4	4	25	6.25
105-8	5%SSA LP1846	2	0	3	2	4	4	4	4	23	5.75
91-6	15%SSA Water	0	0	0	0	0	0	0	0	0	0

* Skin Surface Area

Clinical and Behavioral Observations

As methemoglobin concentrations increased to 10% and above, the high dose animals in both Replicates A and B became increasingly lethargic, generally moving around the pen only to eat and drink. These animals displayed more normal behavior as the methemoglobin concentration returned to baseline. All treated animals showed erythema and developed pustules which progressed to epidermal crusts by day 4. Resolution of the skin lesions was well advanced by day 15.

Body Weights and Food Consumption

The body weights for animals in Replicates A and B are listed in Table 6. All pigs, with the exception of the 7.5% SSAE in Replicate A (1.3% weight loss between day 1 and day 15), gained between 4.3 and 7.7% body weight over the course of the study, although more than half the animals lost slight amounts of weight between day 8 and day 15. All animals consumed their daily allotment of food; the high dose pigs did not consume their daily allotment as quickly as the other animals during those specific times corresponding to high methemoglobin levels (days 2, 3 and 4).

Discussion

The objective of this study was to establish a dose range from which optimal cutaneous applications of LP1846 could be selected for use in the definitive toxicity study in male Hanford Miniature Swine. It was previously established that liquid propellants such as LP1846 are toxic in several species including the miniature swine (1,5). This range-finding study established that topical applications of LP1846 in male Hanford Miniature Swine became absorbed through the skin and resulted in oxidation of hemoglobin to methemoglobin and initiated the formation of Heinz bodies. Two replicate studies were needed to establish the dose range. Data from Replicate A, where SSAEs were 15%, 7.5%, 3.8% and 1.9%, were not sufficient to establish an adequate dose-response curve. The application scheme was modified to 15%, 12.5%, 10% and 5% SSAE for Replicate B. Those applications resulted in methemoglobin concentrations which more closely resembled a dose-related (i.e., percent skin surface area) response.

The 15% SSAEs in both Replicates A and B caused sufficient absorption to result in greater than 20% methemoglobin levels between days 3 and 4 post-exposure, which persisted for approximately 2 days. Because methemoglobinemia will spontaneously resolve over 1 to 3 days following clearing of the inducing agent (6), these observations indicate that the parent compound and/or metabolites of LP1846 remained active for approximately 1 week following a 15% SSAE in male Hanford Miniature Swine. Because lethargy, ataxia, and semistupor usually are not apparent until methemoglobin content reaches 50% (7), it is possible that the lethargy noted under clinical and behavioral observations in the 15% SSAE animals may be related more to the degree of cutaneous irritation than to the methemoglobin levels. Alternatively, the extreme reaction may be related to less well understood systemic effects of LP1846.

Table 6. Body Weights (Kilograms)

Replicate A

	High Dose (15%)	High Middle (7.5%)	Low Middle (3.8%)	Low Dose (1.9%)	Control (15%)
Allocation	28.2	32.0	28.8	30.3	27.8
Day 1	28.4	31.7	28.9	29.8	28.3
Day 8	30.4	31.6	31.7	31.4	30.7
Day 15	30.6	31.3	31.1	31.4	30.4

Replicate B

	High Dose (15%)	High Middle (12.5%)	Low Middle (10%)	Low Dose (5%)	Control (15%)
Allocation	32.9	35.1	34.5	34.6	32.2
Day 1	34.1	35.3	34.9	35.2	33.1
Day 8	35.0	37.3	37.5	37.4	34.3
Day 15	35.9	37.7	36.8	36.7	34.9

Methemoglobin is hemoglobin in which the iron has been oxidized from the ferrous (Fe^{+2}) to the ferric (Fe^{+3}) state, thereby rendering it incapable of transporting oxygen. Estimates are that between 0.5 and 3% of the body's hemoglobin is spontaneously oxidized to methemoglobin daily (8,9). Actions by enzyme systems in the RBC, primarily that of the cytochrome-b_5 reductase system which is the primary enzyme system responsible for reducing methemoglobin to hemoglobin in mammalian RBCs, are normally able to keep the methemoglobin content at about 1% of total hemoglobin (10). Another means that aids in maintaining low in vivo levels of methemoglobin is metabolic activity, such as detoxification of oxidants before their reaction with hemoglobin (11).

Formation of Heinz bodies in mammalian RBCs results from exposure to many oxidative compounds (12). Because methemoglobin formation is also a component of oxidative injury to RBCs, it has been proposed that there is a direct association between elevated methemoglobin production and Heinz body formation (13). The consensus, however, is that the two are not directly related (14). Comparison of the effects of various oxidants shows a lack of correlation between the propensity to form methemoglobin and Heinz bodies. For example, nitrite produces methemoglobin without Heinz body formation or anemia in animals (10).

Unfortunately, the methemoglobin and Heinz body data from the Replicate B animals of this study do not help to resolve the controversy over possible direct association between the two. The percentage of RBCs with Heinz bodies in Replicate B animals decreased even as the concentration of blood methemoglobin increased. In all but the 15% SSAE animal, the percent Heinz bodies remained above 30% at sacrifice (Day 15) even though the methemoglobin concentrations had decreased to below baseline levels. Data from the Replicate B animals also depict a decrease in numbers of circulating RBCs and concomitant increases in numbers of circulating reticulocytes and in RBCs containing Heinz bodies, suggesting physiological removal of injured RBCs and early release of immature RBCs from hematopoietic tissues.

LP1846 is a cutaneous irritant and was scored in the severe category after topical application to shaved skin of male Hanford Miniature Swine. Because the agent was applied undiluted to all non-control animals of each replicate while only varying the exposed surface area, the cutaneous reaction should have been similar with each animal within that replicate. This expectation held true as there was little or no variation in the PCI scores. The LP1846 treated animals did rub exposed areas of skin against the walls of their pens which may have increased the penetrability of the agent and slightly altered the irritancy scores. Histologic examination of agent-treated skin indicated that resolution of the lesions had progressed well up to the time of terminal sacrifice. The epithelium was intact 15 days after treatment in each specimen examined. There were no other detectable gross lesions. Clinical chemistry data indicated no detectable detriment in either renal or hepatic function in any of the animals.

Conclusions

This range-finding study confirmed earlier data that topical application of LP1846 in male Hanford Miniature Swine produces methemoglobinemia, Heinz body formation and decreases in number of circulating RBCs (5). The data from this range-finding study indicate that topical applications of 5% SSAE and greater cause dose-related changes in methemoglobin concentration, percent Heinz bodies, hematocrit, and percent reticulocytes. The percent of RBCs with Heinz bodies was 87% or more for all agent-treated (5% and higher) pigs (for which the measurement was made) by 48 hours post-application. Although the percentage steadily declined over the length of the study, the numbers were still elevated at sacrifice on Day 15. Thus, Heinz body count is not a sensitive indicator of exposure. One should monitor methemoglobin concentration, the more sensitive indicator, following topical exposure to LP1846.

Aside from its hematologic effect, LP1846 causes severe skin irritation when applied topically to shaved skin of male Hanford Miniature Swine. The skin lesion progressed from mild erythema to pustule formation in a matter of 24 hours. The epidermal surface was intact with variable amounts of surface crusting (scab) present at day 15 after exposure.

In conclusion, this range-finding study showed that a positive dose response exists between the skin surface exposed to undiluted LP1846 and methemoglobin levels. Data obtained in this study served as the basis for dose selection for the Phase I study designed to determine the NOEL for methemoglobin formation.

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Chemistry (H. Thompson and his staff): for vitamin C analysis of the bedding;

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Computer Based Systems, Inc. (T. Bixler, A. Taylor, K. Carroll, B. Holland, C. Kolafa, R. Ashcraft, S. Goldman): for support in data collection;

Safety (T. Cole, J. Partridge, B. Hulse, G. Murray): for assistance and support with the many safety concerns and in animal carcass disposal;

Microbiology (C. Cerniglia, B. McClure, J. Knowles and staff): for surveillance of animals during the acclimation stage;

Financial Management Branch (S. Goodrich, S. Sneed) for financial reports for the Quarterly Progress Reports;

Office of Research Services (D. Brand, V. Attwood, J. Appleget, S. Holland): for advice, direction and support in the development and execution of this Interagency Agreement; and

Quality Assurance (J. Reed, M. Albright, D. Taylor, A. Swartzentruber): for advice, guidance and monitoring of the project as per Good Laboratory Practice regulations.

A P E N D I X A

P R O T O C O L

GLP: RAW DATA
Human Subject: _____ Yes XX No
Controlled Sub.: _____ Yes XX No

EXP. NO. E6665.01

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
SHORT-TERM PROTOCOL

TITLE: Dermal Toxicity Range-Finding Study Using LP1846 Gun Propellant on Male Miniature Hanford Pigs

PROGRAM AREA: DOD IAG #224-89-0005

William N. Witt — DATE: 26 Jan 90
Principal Investigator / Division Director

for A Dale Thurman — DATE: 26 Jan 90
Co-Principal Investigator

Robert M. Taylor — DATE: 1/26/90
Co-Principal Investigator

Ronald Cornell — DATE: Jan 26, 1990
Co-Principal Investigator

John F. Young for Fred Kadlubar — DATE: 2/2/90
Assoc. Dir. for Research

George T. Olson Veterinary Services for DBond — DATE: 3/6/90
Assoc. Dir. for Research Services

Stephen M. Knuglas for — DATE: 3/15/90
Assoc. Dir. for Management

William N. Witt — DATE: 15 Feb 90
ACUC Chairman - acting

J. E. Bentley — DATE: 14 MAR 90
Safety Officer

Approved By: See Amendment 2, dated 23 MAR 90 — DATE: _____
Contracting Officer's Representative, USABRDL

Approved By: N/A — DATE: 26 Jan 90
Director, NCTR

TABLE OF CONTENTS

1.0 EXPERIMENT TITLE

Dermal Toxicity Range-Finding Study Using LP1846 Liquid Gun Propellant on Male Miniature Hanford Pigs

1.1 EXPERIMENT NUMBER

E6665.01

1.2 FACILITY

National Center for Toxicological Research
P.O. Box 26
Jefferson, AR 72079

1.3 PRINCIPAL INVESTIGATOR

William M. Witt, DVM, PhD, NCTR

1.4 CO-PRINCIPAL INVESTIGATORS

Robert M. Parker, PhD, Pathology Associates, Inc.
J. Dale Thurman, DVM, MS, Pathology Associates, Inc.
Paul Gosnell, BS, Pathology Associates, Inc.

2.0 OBJECTIVE

To determine the dose levels for a study on the potential for toxicity of dermal applications of LP1846 Liquid Gun Propellant on male Miniature Hanford Pigs using hematological and histopathological assays.

2.1 SPECIFIC AIMS

To determine the Maximum Tolerated Dose (MTD), the appropriate time periods for blood sampling for the methemoglobin measurements, the efficacy of selected clinical chemistry assays using pig serum and to refine the Standard Operating Procedures for blood collection and dosing to be used in subsequent studies with dermal applications of LP1846 Liquid Gun Propellant in male Hanford Minature Pigs.

3.0 BACKGROUND

LP1846 liquid gun propellant is under development by the U.S. Army as a possible substitute for current solid gun propellants. The limited toxicity data that are available on several liquid gun propellant formulations, as well as their major components, indicate

that they are toxic in rabbits, guinea pigs and miniature pigs. The major toxic effects involve the erythrocyte by crenation of cells, Heinz body formation and methemoglobin production. The toxic effects with monopropellants such as LP1846 are apparently due to the ingredient hydroxylammonium nitrate (HAN) and produce a nitrate-type of poisoning. (Extracted from Freedman, An Outline of Liquid Gun Propellants, not dated)

4.0 CHEMICAL COMPOSITION OF LP1846

Ingredients (% by weight):
19.2% TEAN (Triethanolammonium nitrate- $C_6H_{16}N_2O_3$)
60.8% HAN (Hydroxylammonium nitrate- $N_2H_4O_4$)
20.0% H_2O
Molecular weight: TEAN- 164; HAN- 96
Physical data: Liquid, white to very pale straw colored, oderless
Stability: No decomposition within 48 hours at 70°C
Density (g/cc): 1.42
Viscosity (@0°C): 25 cp
(Extracted from Freedman, An Outline of Liquid Gun Propellants, Not dated)

4.1 DOSE LEVELS

The dose solution will be neat LP1846 Liquid Gun Propellant provided by the Sponsor. The actual dose level will be calculated from an application to the first pig. The volume of LP1846 required to cover approximately 15% of skin surface area will be determined.

4.2 CHARACTERIZATION OF THE TEST ARTICLE

Characterization of the LP1846 Liquid Gun Propellant will be performed by the Sponsor (US Army Armament Research and Development Command, Aberdeen Proving Ground, Maryland). No purity analysis will be done at the NCTR.

4.3 CONTROL ARTICLE

The control article will be distilled water.

4.4 ANIMAL HOUSING

Acclimation Location - Building 14 rooms 101, 103
Animal Treatment Location - Building 14 rooms 102, 104
Material of Runs - Brushed aluminum
Acclimation housing: 6' x 18' (group housing for 5 animals)
Experimental housing: 3' x 6' (individual housing)
Bedding Material: Pine shavings (Northeastern Products Corporation)

Temperature: 23°± 3°C
Relative Humidity: 50 ± 10%
Light/Dark Ratio: 12 hr/12 hr
Clinical Surveillance: Daily
Bedding Changes: Daily around water source; twice-weekly for entire run
Feed: Purina Commercial miniature pig diet
Frequency: once per day (1 kilogram allotments)
Contaminants in Feed That are Expected to Interfere with Study: None
Water: Filtered
Frequency: *Ad Libitum*
Contaminants in Water That are Expected to Interfere with Study: None

4.5 ANIMAL MODEL

Species/Strain: Hanford Miniature Pig (Charles Rivers)
Sex: male
Age: 4-5 months old at delivery
Weight: 21-27 Kg at delivery
Procedure for Individual Animal Identification: Supplier identification will be used.

Young adult male Hanford Miniature Swine (Charles Rivers) will be purchased and shipped to NCTR and will be held for a 14-day acclimation period. Physical examinations, base-line hematology/microbiology/clinical chemistry and conditioning to restraint slings will be performed during the acclimation period.

5.0 EXPERIMENT DESIGN

Protocol Duration: 15 Days
Route of Administration: Dermal application (topical application to skin)
Frequency of Administration: Single Dose
Dose Levels (mg LP1846/Kg body weight): To be determined
Number Treated Animals Required/Dose Level: 1 male (4 males total)
Number Control Animals Required: 1 male
Number of Animals Required for Possible Replicate: 5 additional males
Weighing Schedule: Day of treatment, then weekly, and at sacrifice
Biologic Sample Requirements: Blood samples (on day of allocation, day 1 Time 0, 2h, 4h, 8h, and 24h and once daily days 2 through 14 and at necropsy (day 15).

The Rangefinding will use a total of 5 animals per replicate (two replicates may be required). The animals will be randomly assigned, one to each treatment and control. The exposure will consist of a single topical application of neat agent to the skin. The exposed skin will neither be covered, wiped, nor washed once the agent is applied. The exposure area for the control and high dose pigs will encompass approximately 15% of skin area. Skin area to be exposed for each animal shall be calculated using the following formula:

$$\text{Body Surface Area } (cm^2) = K \times BW^{2/3}$$

Where: K = 9.95 and BW = Body Weight in grams

The upper-middle, low-middle and low dose pigs will receive that amount of neat agent to cover 7.5, 3.8 and 1.9% of the surface area (approximately one-half, one-fourth and one-eight the calculated high dose volume), respectively. All dosing will be accomplished with the animals in a restraint sling (Charles Rivers) as previously described by Panepinto *et al* (1983). The animals will remain in the sling 4 hours post-exposure at which time they will be placed in individual runs. The animals will be returned to the slings only for blood sampling and physical examination during the remainder of the 14 day test period. The highest dose level to be selected for Phase I of the definitive toxicity test will be the lowest level that produces Observable Adverse Effects (i.e., lethality, morbidity, excessive skin irritation). This dose level will be termed the Maximum Tolerated Dose (MTD). The blood sampling intervals will be adjusted if necessary to establish the time to maximum methemoglobin level and the time required for recovery to the background methemoglobin level in an effort to accurately follow the kinetics of this process. The animals will be observed at least four (4) times daily for clinical signs of toxicity. Skin irritation will be graded according to modification of the method of Draize (1944) (see attached SOP). Body weights will be obtained on the day of exposure, and at sacrifice.

5.1 PATHOLOGY

Methemoglobin levels will be determined directly from whole blood immediately after sampling using an IL282 Co-Oximeter (Instrumentation Laboratory Inc., Lexington, MA). Hematology will be done on days 1-5, 8-12 and 15 and will include a complete blood cell count, including cell morphology assessment, hemoglobin concentration, hematocrit, mean corpuscular volume, % red cells with Heinz bodies, and, starting 3 days post-exposure, reticulocyte counts. Clinical chemistry samples to determine the pig liver and kidney function (BUN, total protein, creatinine, albumin and ASAT) will be collected on days 1 - 4, 8 and 15. Gross necropsies will be performed on all moribund animals and animals which die on test. At the termination of the study (day 15), all surviving animals will be euthanized by *iv* injection of T-61 Euthanasia Solution (0.3 ml/Kg) and necropsied. Histopathology will be performed on sections of skin exposed to the test and control articles (both representative samples and lesions occurring in these areas), as well as on any gross lesions observed during the necropsies. An additional skin tissue sample will be collected from a site that is approximately 5.0 cm away from the edge of the treatment area.

5.2 DATA COLLECTION/RECORDS TO BE MAINTAINED

Data collection (body weights, clinical observations, mortality data and day (date) of sacrifice) is to be entered on the INLIFE automated data collection system. Dosing data (body weight and dose volume delivered) will collected by the INLIFE Dosing Program.

Data acquisition, retrieval, reports and management support will be provided by DIMS. The Draize Test records and any other manually recorded data will be maintained by the Principal Investigator.

6.0 QUALITY ASSURANCE

This protocol also meets the United States Environmental Protection Agency Guidelines for the Health Assessment of Suspect Developmental Toxicants: Final Rules (1986) [Federal Register, 51(185): 34028 - 34040] and the United States Environmental Protection Agency Toxic Substances Control Act Test Guidelines: Final Rules (1985) [Federal Register, 50(188): 39426 - 39436]. All aspects of the studies will be conducted in accordance with Good Laboratory Practice Regulations, Food and Drug Administration (Federal Register, Vol. 52, No.172, September 4, 1987, pp. 33768 - 33782). All aspects of the studies will be conducted in accordance with written Standard Operating Procedures (SOP) of the performing unit. An administratively separate quality assurance unit (QAU) at NCTR will monitor the studies to assure adherence to good laboratory practices and the approved SOPs.

7.0 STORAGE OF RECORDS

All hard copy of data sheets for the present study will be stored in the NCTR Archives under the control of the NCTR Quality Assurance Officer. Biological samples collected during the course of the study will be placed in secure storage in the Pathology Division, NCTR. Work sheets and computer printouts generated in the statistical analysis of data will be stored at the NCTR. In accordance with Sections 58.190 and 58.195 of the Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (1987), all records, data and reports will be maintained in storage for a minimum of five years; biological samples will be maintained for a minimum of five years or for as long as the quality of the preparation affords evaluation, whichever is less. These parameters meet the request of the sponsor that the storage of records will be done in accordance with EPA Good Laboratory Practice Standards 160.195(b3).

8.0 PERSONNEL

Principal Investigator: William M. Witt, DVM, PhD
Co-Principal Investigator: Robert M. Parker, Ph.D.
J. Dale Thurman, DVM, MS
Paul Gosnell, BS
Clinical Chemistry: Linda Harbour
Surety Facility Manager: Paul Gosnell
The Bionetics Corporation Personnel: Bob Harmon, Supervisor
Elijah Smith, Technical Specialist
Other personnel will be identified in the study file.

9.0 CARE AND USE OF LABORATORY ANIMALS

This protocol will be conducted in accordance to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 85-23, Revised 1985). The NCTR Animal Care and Use Committee will monitor animal care issues of this protocol.

10.0 SIGNIFICANCE AND BENEFITS

LP1846 liquid gun propellant is under development as a possible substitute for current solid gun propellants. Limited toxicity data are available on several liquid gun propellant formulations as well as their major components. Data confirms that they are toxic in rabbits, guinea pigs and miniature pigs. The dermal toxicity assay is an ***in vivo*** test that would provide toxicologic information about the risk from dermal absorption of LP1846.

11.0 TIME FRAME

Study #	Select	Start	End Study
E6665.01	**TBA**	**TBA**	**TBA**

12.0 REFERENCES

1. Freedman, Eli, ed. An Outline of Liquid Gun Propellants, USAARD, Aberdeen Proving Ground, MD.

2. Draize, J.H. *et al.* Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther., 82:377-390 (1944).

3. Panepinto, L.M. *et al.* A comfortable, minimum stress method of restraint for Yucatan miniature swine. Lab. Anim. Sci., 33:95-97 (1983).

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Standard Operating Procedure

TITLE: EXAMINATION OF PIG SKIN FOR PRIMARY IRRITATION FROM TOPICAL APPLICATION OF LP1846 LIQUID GUN PROPELLANT.

PURPOSE: TO ESTABLISH A PROCEDURE FOR GRADING THE CUTANEOUS-IRRITATION POTENTIAL OF LP1846 LIQUID GUN PROPELLANT.

GENERAL DIRECTIONS:

1) All clinical evaluations of cutaneous irritation will be made with the animal resting in a restraint sling. The evaluations are to be made and recorded for each animal at 4, 24, 48 and 72 hours post treatment with LP1846 Liquid Gun Propellant.

2) Grade the exposed area according to the following table:

SCORING SYSTEM

SKIN REACTION	SCORE
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined but mild erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formations (injuries in depth)	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised ~ 1mm)	3
Severe edema (raised > 1mm and extending beyond the area of exposure)	4

3) Scoring Procedure. The treated area is scored for erythema and edema using the numerical scale (0-4 in each case according to severity) provided in the above table. The sum for each reading (score for erythema and score for edema added together for each time period) is to be totaled after the 72 hour reading and divided by 4 (the total number of evaluation periods) to provide a mean score termed the "primary cutaneous irritation index (PCI).

4) Interpretation. The irritancy of the LP1846 Liquid Gun Propellant will be defined as:
   a. PCI below 0.5 - non-irritant
   b. PCI 0.5 - 3 - slightly irritant
   c. PCI 3-5 - moderately irritant
   d. PCI 5-8 - severely irritant

SPECIFIC DIRECTIONS:

1) Place animal in restraint sling being careful not to disturb the test site.

2) Score the cutaneous irritation of the test site as instructed in the above General Directions.

3) Record the score on the appropriate form (to be developed)

4) Return the animal to its individual run.

William M. Witt
Principal Investigator

2/8/90
Date

Don Brandt
Project Officer

2/8/90
Date

LABORATORY ANIMAL CARE AND USE FORM

National Center for Toxicological Research

Experiment Number 6665.01

Title: Dermal Toxicity Range-Finding Study Using LP1846 Liquid Gun Propellant on Male Hanford Pigs

Investigator(s): Witt, Parker, Thurman & Gosnell Ext. 4949

I. ANIMAL SPECIES & HOUSING:

A. Animals:

		Species	Strain	Sex	Age/Wt.	No.	Source
Fiscal Year 1	a.	Miniature Swine	(Hanford)	M	4-5mo (21-27Kg)	10	Charles Rivers
	b.						
	c.						
Fiscal Year 2	a.						
	b.						
	c.						
Fiscal Year 3	a.						
	b.						
	c.						

B. Are there special housing, diet, environment or other procedural requirements? If so, describe: Housing in Bldg 14, Rooms 101, 102, 103 & 104, which have been renovated for the purpose of housing swine. Animal husbandry requirements will be covered in start up meetings

Other special instructions: _____

Sick animal disposition [X] Notify investigator [ ] Pathology [ ] Microbiology [ ] Euthanasia

Technical Service needs [X] Transportation [ ] Injections [X] Sampling [X] Weights/Observations [X] Daily moribund/death check and frequency 4 times per day for first 4 days of exposure, then 2 times per day to study end.

Other _____

II. RATIONALE for involving animals and the appropriateness of the species (and strain if applicable) and numbers used.

The study is covered by an Interagency Agreement with the Department of Defense. The male miniature Hanford pig was selected as a model due to: ① the sponsor's request; ② the permeability of pig skin is the closest animal model to human skin (Bartek, et al.); and ③ the preliminary studies have been completed using miniature swine.

This range-finding study will use 5 animals per replicate (4 treatment animals and 1 control [negative] animal) with a possibility of using 2 replicates (total of 10 animals). Several of the animals will first be used to refine husbandry Standard Operating Procedures (SOPs) to be used in the follow-on definitive toxicity studies.

Reference: Bartek, M.J., LaBudde and H. Maibach. Skin Permeability in vivo: Comparison in Rat, Rabbit, Pig and Man. J of Investigative Dermatology, Vol 58 #3, pp 114-123.

III. ABSTRACT OF PLANNED USE OF ANIMALS

Write a brief yet complete description of all animal-related methodology in laymen's terms. Use additional sheets as necessary.

See Attached sheet.

IV. CATEGORIES of experimentation based upon level of manipulation and pain. Please circle category:

A. Live animals will be humanely killed without any treatments, manipulations, etc., but will be used to obtain tissue, cells, etc., or live animals will be exposed to treatments which do not induce significant discomfort or distress (e.g., simple procedures such as injections of acutely relatively harmless substances, blood sampling, etc.).

B. Live animals will have significant manipulations, surgery, etc., performed while anesthetized. The animals will be humanely killed at the termination of the procedure without regaining consciousness.

(***C) Live animals will be subjected to SHORT duration pain or discomfort. Such procedures cause post-anesthetic pain/discomfort (i.e., (catheters), implants, surgical wounds/incisions). Also included are immunization with Freund's Adjuvant, noxious stimuli from which escape is possible, mild toxic drug or (chemical exposure), tumor implants/hybridomas, tethered animals, (short-term restraint), mother/infant separations. Category C procedures incur additional concern in proportion to the degree and duration of unavoidable stress or discomfort.

***D. Live animals will be subjected to significant but UNAVOIDABLE stress or pain; + analgesics to be provided as necessary. Deliberate induction of behavioral stress, induction of anatomical or physiological deficits resulting in pain or distress, noxious stimuli from which escape is impossible, prolonged periods of physical restraint, major surgical procedures under anesthesia resulting in significant post-operative pain. Category D experiments fall into Category E, below, if anesthesia, analgesia or tranquilizers are not utilized, where appropriate, to minimize pain or stress.

***E. Live animals will be subjected to significant but UNAVOIDABLE stress or pain + without the benefit of anesthesia, analgesics or tranquilizers. Examples in this category include all those in Category D above and procedures which produce pain in which anesthetics are not used, such as toxicity testing with death as a probable end point, radiation sickness, irritants, burns, trauma, biological toxins, severe climatic stress, long-term restrictions in food/vater intake, drug addiction, and stress and shock research that would result in pain approaching the pain tolerance threshold, i.e., point at which intense emotional reactions occur. Category E experiments present an explicit responsibility on the investigator to explore alternate designs to ensure that animal distress is minimized.

***Complete Section V.

+ Consult with Division of Veterinary Services.

V. DESCRIPTION OF ANIMAL CARE:

Non-surgical procedures [for completion if items IV, C - E were circled].

A. Give signs of disease/illness that may be expected to occur as a result of your study, if any: Excess levels of methemoglobinemia may result in the high dose animal(s). Such conditions may cause weakness, staggering gait, collapse, dyspnea, cyanosis and rapid pulse. Death may result in severe cases. Skin irritation may result from the dermal (topical) application of the test article.

B. Give methods of treatment/care that may be provided by either your staff, Colony Support Veterinarians or Animal Husbandry Contractor personnel which can alleviate signs observed. If treatment is contraindicated for your results, please so state. Induction of methemoglobinemia is an objective of the study and therefore, no treatment which might alter the production of methemoglobin may be given.

C. May analgesics be used? Should excessive skin irritation result from the topical application of the agent, the use of topical analgesics will be utilized where needed.

D. May moribund animals be euthanized? [After veterinary consultation with PI] Yes, after consultation with PI/CO-PI.

E. What other special care/handling is necessary for your animals? Any special handling of the miniature swine will be coordinated with the animal care contractor during start-up meetings and will be covered by SOPs.

VI. SURGICAL PROCEDURES (if any): (Possible use of vascular indwelling catheters for collection of blood samples), will amend the LACUF if the procedure is required. MW

A. Survival* _____ Non-survival _____
*All survival surgery will be performed under aseptic conditions.

B. Where is surgery to be performed? _____

C. Person performing surgery and experience in performing these procedures:

_____

_____

D. Briefly describe surgical procedures: _____

_____

_____

E. Anesthetic regimen:

Drug(s) _____ Dose _____

Route _____ Duration _____

Who will administer/monitor anesthesia and what are their qualifications?

_____

_____

If anesthetics are not used, justify _____

_____

_____

F. Post-operative pain or distress:

Will analgesics/tranquilizers be used for post-operative pain?

_____ Yes _____ No

Drug(s) _____ Dose _____

Route _____ Duration _____

If analgesics/tranquilizers will not be used, justify _____

_____

_____

G. Post-operative care:

1. Intensive care required? _____ Yes _____ No

What time period? _____

Who will provide care? _____

2. Post-operative routine:

Who will provide? _____

What monitoring will be done? _____

What drugs/chemicals administered? _____

Antibiotics? Type/dosage/frequency _____

Special care to be provided _____

PERSON(S) TO CONTACT IN AN EMERGENCY _____

_____ Phone (office) _____

_____ Phone (home) _____

3. What post-operative complications may be expected? _____

_____

4. Any contra-indications? _____

5. Will individual animals be subjected to more than one major surgical procedure? ____ Yes ____ No

Explanation/justification _____

VII. EUTHANASIA

What is/are method(s) of euthanasia?

i.v. injection of T-61 euthanasia solution (0.3ml/kg)

Method(s) in compliance with AVMA Panel on euthanasia recommendations?

X Yes ____ No

If not, explanation/scientific justification _____

Name and qualifications of person(s) performing euthanasia William M. Witt, DVM, PhD and J. Dale Thurman, DVM, MS - both are veterinarians and pathologists experienced in euthanasia techniques in a wide variety of spp.

VIII. BIOHAZARDS OR RADIOISOTOPES USED? X Yes ____ No

A. If Yes: Chemical X Biological _____ Radiation _____

B. List of hazardous substances: LP 1846 Liquid Gun Propellant

Have the appropriate safety offices been notified and precautions taken?

X Yes ____ No

IX. PRINCIPAL INVESTIGATOR ASSURANCE:
The information contained herein is accurate to the best of my knowledge. Procedures involving animals will be carried out humanely and all procedures will be performed by or under the direction of trained or experienced persons. Any revisions to animal care and use in this project will be promptly forwarded to the ACUC for review. Revised protocols will not be used until ACUC approval is received. The use of alternatives to animal models have been considered and found to be unacceptable at this time.

William M. Witt — Signature

8 January 1990 — Date

THIS PROTOCOL HAS BEEN APPROVED FOR ANIMAL CARE AND USE.

William M. Witt — Chairman, Animal Care and Use Committee

15 February 1990 — Date

III. ABSTRACT OF PLANNED USE OF ANIMALS

The Rangefinding will use a total of 5 animals per replicate (two replicates may be required). The animals will be randomly assigned, one to each treatment and control. The exposure will consist of a single topical application of neat agent to the skin. The exposed skin will neither be covered, wiped, nor washed once the agent is applied. The exposure area for the control and high dose pigs will encompass approximately 15% of skin area. Skin area to be exposed for each animal shall be calculated using the following formula:

$$\text{Body Surface Area } (cm^2) = K \times BW^{2/3}$$

Where: K = 9.95 and BW = Body Weight in grams

The upper-middle, low-middle and low dose pigs will receive that amount of neat agent to cover 7.5, 3.8 and 1.9% of the surface area (approximately one-half, one-fourth and one-eight the calculated high dose volume), respectively. All dosing will be accomplished with the animals in a restraint sling (Charles Rivers) as previously described by Panepinto *et al* (1983). The animals will remain in the sling 4 hours post-exposure at which time they will be placed in individual runs. The animals will be returned to the slings only for blood sampling and physical examination during the remainder of the 14 day test period. The highest dose level to be selected for Phase I of the definitive toxicity test will be the lowest level that produces Observable Adverse Effects (i.e., lethality, morbidity, excessive skin irritation). This dose level will be termed the Maximum Tolerated Dose (MTD). The blood sampling intervals will be adjusted if necessary to establish the time to maximum methemoglobin level and the time required for recovery to the background methemoglobin level in an effort to accurately follow the kinetics of this process. The animals will be observed at least four (4) times daily for clinical signs of toxicity. Skin irritation will be graded according to modification of the method of Draize (1944).

Body weights will be obtained on the day of exposure, and at sacrifice

Methemoglobin levels will be determined directly from whole blood immediately after sampling using an IL282 Co-Oximeter (Instrumentation Laboratory Inc., Lexington, MA). Hematology will be done on days 1-5, 8-12 and 15 and will include a complete blood cell count, including cell morphology assessment, hemoglobin concentration, hematocrit, mean corpuscular volume, % red cells with Heinz bodies, and, starting 3 days post-exposure, reticulocyte counts. Clinical chemistry samples to determine the pig liver and kidney function (BUN, total protein, creatinine, albumin and ASAT) will be collected on days 1 - 4, 8 and 15. Gross necropsies will be performed on all moribund animals and animals which die on test. At the termination of the study (day 15), all surviving animals will be euthanized by *iv* injection of T-61 Euthanasia Solution (0.3 ml/Kg) and necropsied. Histopathology will be performed on sections of skin exposed to the test and control articles (both representative samples and lesions occurring in these areas), as well as on any gross lesions observed during the necropsies. An additional skin tissue sample will be collected from a site that is approximately 5.0 cm away from the edge of the treatment area.

B. Toxic/Hazardous Material Use Request

NOTE: This form must be completed by the PI and attached to the study protocol. Please contact the Safety Staff regarding questions or assistance concerning this form (NCTR Form SS-6 12/87).

Study Information:

Experiment #: 6665.01 PI witt

Division: Veterinary Services

Experiment Name Dermal Toxicity Range-Finding Study Using LP1846 Liquid Gun Propellant on Male Miniature Hanford Pigs

Chemical Information:

Name: LP1846 Liquid Gun Propellant* CAS No. N/A

Supplier: US Army Armament Research and Development Command

Quantity to be Procured: 1.5 liters Physical State: Liquid

Radioactive Material: Yes ___ No X Isotope: ___

Controlled Substance: Yes ___ No X Class: ___

Material Safety Data Sheet (MSDS) attached: Yes X No X

2/8/90

* ~~Requested from the Army and is being sent to the P.I.~~

Use Conditions:

Storage Location: Bldg 37 Safety Storage Use Location: Bldg 14, RM 101 and 103

Experimental Procedures: (Briefly describe in general terms how material will be used).

Undiluted agent (LP1846 GUN PROPELLANT) will be topically applied to the skin of miniature pigs. There will be a one-time exposure and the agent will not be removed following exposure.

* Active ingredients : 19.2% TEAN (Triethanolammonium nitrate)
60.8% HAN (Hydroxylammonium nitrate)

Special handling procedures and personal protective equipment required:

The agent is a Class B Explosive and has no decomposition within 48 hours at 70°C. There is limited evidence that the agent can cause contact dermatitis following topical exposure. The agent is readily absorbed through the skin and can result in methemoglobinemia. Tyvek suits, impervious gloves, safety glasses and rubber boots will be provided personnel handling the agent.

Describe decontamination and waste disposal procedures to be used:

Decontamination procedure is a thorough wash down with water. Bedding and wastes will be incinerated by the NCTR Safety staff.

Emergency procedures (in the event of overt personnel exposures):

Immediate and thorough rinsing of the area exposed with water; report to the NCTR Health clinic to be monitored for methemoglobinemia and referral for possible treatment of same.

Names of personnel or support groups involved in handling this material:

William M. Witt, HFT-240, EXT 4949
J. Dale Thurman, PAI-923, EXT 4670
Paul Gosnell, PAI-923, EXT 4034
NCTR SAFETY, HFT-2, EXT 4388/4418

APPROVED: _____

FAX HEADER SHEET

TO: Bill Witt

ADDRESS: NCTR

FAX: 1-501-591-4030

FROM: Jody Waszczowski

ADDRESS: USA BALLISTIC RESEARCH LABORATORY
SLCBR-IB-B
ABERDEEN PROVING GROUND, MD 21005-5066

PHONE: 301-278-6160

FAX: 301-278-6159

NO. OF PAGES (INCLUDING HEADER): 10

COMMENTS: I understand that our shipping dept. picked up your material 18JAN1990. It should have left here within the next week. It's arrival is imminent. I will forewore assays within the next day or two sorry for the delay.

OCCUPATIONAL HEALTH GUIDE FOR HYDROXYLAMMONIUM NITRATE (HAN) AND LIQUID PROPELLANT FORMULATIONS CONTAINING HAN

1. Medical Surveillance.

a. Preplacement or baseline. Occupational and medical histories with attention to the cardiovascular, hematologic and dermatologic systems. Physical exam with emphasis on the cardiovascular system, skin, liver and spleen. Laboratory exam to include complete blood count with indices and morphology, hemoglobin, and hematocrit.

b. Periodic. The above mentioned exam should be performed on an annual basis.

c. Termination. The above mentioned exam should be performed on termination of the employee if not done in the past six months.

2. Precautions.

a. Safeguards to prevent ingestion, inhalation or skin contact are mandatory. Eating, drinking, and smoking must not be permitted in areas where HAN or HAN-based propellants are handled or stored. Wearing of personal protective equipment (protective clothing, rubber gloves, and chemical splash goggles) is required. Clothing contaminated with HAN or HAN-based propellants must be removed immediately. Contaminated skin or eyes should be flushed immediately with large volumes of water. Workers must shower after work and before changing into street clothes. When exposure to aerosols or excessive vapor concentrations is likely to occur, respiratory protection is required.

b. SOPs should be written governing safe use. Information concerning this hazard should be incorporated into a health education program.

3. Treatment.

a. Signs and symptoms of acute overexposure to HAN may include headache, nausea, vomiting, flushing of the skin, marked drop in blood pressure, cyanosis (bluish discoloration of skin), shortness of breath, convulsions, collapse, or unconsciousness.

b. Despite Materiel Safety Data Sheet statements to the contrary, experimental evidence from pig dermal toxicity studies suggests rapid absorption of HAN-based propellant through the skin resulting in systemic toxic effects (methemoglobinemia and Heinz body formation). In the event of skin contamination, immediately remove any contaminated clothing and flush with large volumes of

water. If inhaled, remove the victim to fresh air. If ingested, induce vomiting with ipecac, if available, and victim is conscious.

c. Seek medical attention immediately if exposed and symptomatic. Medical personnel should be aware of the potential for hypotension and methemoglobinemia in the highly exposed worker. Treatment of patients with methemoglobinemia should be individualized, depending on the severity of clinical manifestations. For mildly affected individuals, removal from exposure may be the only treatment necessary. Severely hypoxemic patients with methemoglobinemia may require intravenously administered methylene blue (1-2 milligrams per kilogram body weight), at the discretion of the treating physician.

SAFETY DATA AND INFORMATION ON HANDLING OF HAN-BASED LIQUID PROPELLANTS AND THEIR COMPONENTS

PURPOSE - To establish safe operating procedures and identify hazards in the handling of Liquid Propellants (LP) in a laboratory.

SCOPE - This summary applies to laboratory safety procedures for handling HAN-based LP's and their components. Any hazardous operation, experiment, equipment not covered within this document, should be addressed by more specific documentation. Laboratory equipment should be operated in accordance with manufacturer's instructions and all routine laboratory safety and health provisions must be adhered to. All personnel must be made aware of hazards prior to working with liquid propellants.

BACKGROUND - HAN-based LP's are monopropellants composed of an oxidizer, HAN (hydroxylammonium nitrate), and a fuel, an aliphatic amine nitrate. These propellants are formulated to be stoiciometric with respect to CO_2, H_2O and N_2. They are electrically conducting ionic solutions, therefore no static charge can be built up. Almost all vapors from the propellants are water vapors. The propellant can fume off if heated, but does not burn unless pressurized. Any oxidizing or reducing agent will react with HAN-based propellants. These propellants will complex with most heavy metals and decompose. If confined, this decomposition could progress to a violent reaction. HAN-based LP in it's pure state looks like water, so any coloration indicates a reaction and that propellant should be handled with extreme caution. Partial decomposition of LP produces toxic NOx compounds.

STORAGE - (See Hazards) Storage of liquid propellants or components in a laboratory should be limited to the following:

LP 1845 or LP 1846 - 30 cc
HAN - 100 cc
Amine Nitrate Salts - 50 g each.

None of the above items may be stored together and all must be stored in a fume hood. These items should not be exposed to heat, open flame or sparks. Conducting floors are not required.

HANDLING - 1. Protective Clothing - Use of polyethylene gloves is required. Safety glasses must be worn when handling the propellants or components. Laboratory coats should be worn if splashing of propellant is possible or propellant is under any pressure. As specified later, work clothing must be changed if exposed to propellant or components and material should be thoroughly washed prior to disposal or reuse, to prevent fire hazards. Every effort must be made to reduce dermal exposure.

2. Laboratory Equipment - Liquid propellants will react with most metals and other common materials. The following is a list of material that are acceptable for use with these items:

- polyethylene
- polypropylene
- polytetrafluoroethylene (Teflon™)
- borosilicate glassware
- common ceramics (alumina, magnesia, berylia)
- ANSI 316 and 413 stainless steel

The following is a list of materials known to react with the propellants and must NOT be used:

- ANSI 303 and 304 stainless steel
- nickel-based alloys (e.g. Nichrome)
- aluminum, magnesium and their alloys
- polyvinyl chloride (e.g. Tygon™)
- some rubbers (curing agents may react with LP)
- polycarbonates

DISPOSAL - All propellants and components are ionic nitrate salts and are water miscible and biodegradable, therefore they can be disposed of by dilution and conventional sewage. Dilution must be at least 10:1, ten parts water to one part propellant (similar to acids) and sewage should be closed loop biodegradable. Any combustible material (cloth, paper) contacting the propellants should be washed thoroughly prior to disposal to prevent fire.

FIRE PROTECTION - As above, all items are water miscible therefore, available fire protection should be water deluge.

HAZARDS - Fire or Explosion -

Vapor Reactions - These items are aqueous solutions of two salts. The salts form ionic solutions and almost all of the vapor is water vapor. Therefore, vapors do not interact with heat or flame to produce a dangerous condition.

Heat Reactions - Propellants or components should not be exposed to temperatures greater than 60 deg. C as higher temperatures may cause the propellant to fume off. Exception: experiments requiring higher temperatures may be performed in well controlled situations.

Pressure Reactions - Propellant should be stored in thin wall containers that will vent at 15 psi gauge, that is 15 psi above atmospheric pressure (e.g. DOT 12P80 vented closure). This will prevent vigorous reaction initiation at high pressures.

Chemical Reactions - Contact of the propellant with acids or bases must not be allowed.

HAZARDS - Health - The following are guidelines established by the work completed on toxicity testing of these items. Testing

to date has been performed solely on animals, no humans.

LP-1845 and LP-1846 are considered toxic as far as ingestion, dermal contact and inhalation. The principal personal hazards associated with HAN-based liquid propellants are as follows:

a. Contaot of the liquid in the eyes.

b. Exposure of the skin to the liquid.

o. Inhalation of an aerosol vapor.

Toxioity

OCULAR: Application to the eyes produoes mild irritation and pain.

ORAL: The short term toxio response to oral ingestion of LP-1845,46 is methemoglobin formation and a reduction in the oxygen carrying capacity. This results in respiratory distress and cyanosis. Long range tests showed signs of splenic congestion and reticuloendothelial hyperplasia. Results such as anemia, anorexia, myloid hyperplasia of the bone marrow and degenerative changes such as B and T cell atrophy in the spleen were seen in the higher dose levels.

DERMAL: Dermal tests resulted in a high inoidence of chronic and ulcerative dermatitis. Heinz body formation and red blood oell destruction was seen in the higher dose levels.

INHALATION: Inhalation of aerosol was seen to produce the following effects in tests; weight loss, spleen and liver enlargement, respiratory irritation and blood dyscrasia. Minimal effects were seen at low dose levels.

Exposure Limits

Maximum allowable workplaoe airborne concentration of HAN is recommended at 3 mg/m3. Caution: the effeots of skin exposure and inhalation are additive.

First Aid and Self Aid

Accidental ingestion requires water intake (at least two glasses) and induoed vomiting. In the event of skin contamination, flush immediately with large volumes of water. Abrasive soap should be avoided as it may increase the absorption through the skin. Work clothing must be changed immediately if contaminated with HAN. Remove to fresh air and call a physician if expcsed to decomposition products.

Speoial Medioal Information

In the event of accidental ingestion, standard methylene blue treatment for methemoglobinemia (as diagnosed by a physician) is indicated.

SAFETY MEASURES

Detailed written procedures shall be employed for all operations involving propellants. The safety measures pointed out here are only guidelines for the development of standard operating procedures peculiar to each installation.

Education of Personnel

The following subjects should be explained thoroughly to all persons concerned with the handling, storage or transfer of HAN-based liquid propellants:

a. Nature and properties of LP, including the necessity of avoiding contamination

b. Compatible materials of construction

c. Approved handling equipment and its operation

d. Suitable personal protection equipment for handling operation and care of protective equipment. (Clean protective clothing and equipment are essential.)

e. Fundamentals of first aid and self aid treatment for exposure to HAN-based liquid propellant.

Trained supervision of all potentially hazardous activities involving LP is a necessity.

PERSONNEL PROTECTION

The principal hazards for which personal protection should be provided are as follows:

a. Exposure of the body or eyes to the liquid or aerosol vapor

b. Inhalation of the aerosol vapor

c. Exposure to decomposition products (NOx compounds).

Other Safety Equipment

Safety showers and eye wash stations must be available in all work areas where LP is to be handled. Respirators should be provided if the airborne concentration could exceed the maximum exposure limit.

Morton Thiokol, Inc.
Elkton Division
P.O. Box 241
Elkton, Maryland 21921-0241
Emergency Phone (301) 398-3000

MATERIAL SAFETY DATA SHEET

MSDS NO. 111
Date Issued 10/12/87
Written By R. L. Jenkins
Approved By

I. PRODUCT IDENTIFICATION

A. Trade Name and Synonyms: Liquid Gun Propellant, LGP 1846

II. PHYSICAL DATA

A. Appearance and Odor: Water white to very pale straw colored solution. Odorless.
B. Volatiles: Water.

III. COMPOSITION

Hazardous Ingredients	Percent	OSHA PEL	ACGIH TLV
A. Hydroxylammonium Nitrate	61	--	NE
B. Triethanolammonium Nitrate	19	--	NE
C. Water	20	--	--

IV. HEALTH HAZARD DATA

A. Threshold Limit Value: Not established.
B. Effects of Overexposure:
1. Respiratory: None known. Decomposition products are known to cause breathing difficulty and respiratory damage.
2. Eyes: Corrosive liquid effect, moderately severe eye injury if contacted with eyes. Decomposition products can be irritating.
3. Skin: Acidic, pH = 4, corrosive liquid effect, no known dermatitis.
4. Skin Absorption: No absorption observed.
5. Ingestion: Corrosive liquid effect may cause nausea, abdominal discomfort, and collapse. Decomposition products are also corrosive to internal organs.
6. Other: None currently known.

NE = Not Established

MSDS 111
LGP 1846

V. EMERGENCY AND FIRST-AID PROCEDURES
A. Inhalation: If decomposition products are inhaled, remove victim to fresh air. Call a physician and/or emergency facility immediately.
B. Eyes: Immediately flush eyes with large amounts of water for at least 15 minutes. Call a physician and/or emergency facility immediately.
C. Skin: Contact with the skin should be treated as with any corrosive material. Immediately flush the area with water. Wash with soap and water.
D. Ingestion: Accidental ingestion requires water intake (2 glasses) and induced vomiting.

VI. FIRE AND EXPLOSION HAZARD DATA
A. Liquid gun propellant is a DOT Class B (1.3) liquid explosive (Reference: ARBRL-CR-00454, May 1981.) The material does not have a measureable flash point but does have an autoignition temperature (Setchkin) of 310 degrees C (590 F). No explosion occurs in an open fire test and the liquid survives 48 hours at 75 degrees C (167 F). The liquid does not explode under impact of a 4.4-lb weight at 28 inches in a standard cavity test and also does not yeild a positive card gap test at 70 cards.
B. Explosive Limits: DOT Class B explosive (1.3 hazard symbol).
C. Extinguishing Media: Do not attempt to fight burning propellant. Water, CO_2, or foam may be used to restrict spreading of fire after bulk of propellant has burned.
D. Special Fire Fighting Procedures: Propellant ingredients contain oxidizer and fuel. Do not fight fire. If ignited while contained, thrust created while burning may give this propellant uncontrollable ballistic properties. Fire fighting should be limited to preventing the spread of other fires.
E Explosion Hazards: Static discharge, impact, friction, and pinch points between hard surfaces can initiate propellant fires and should be avoided. See VII, B.

VII. REACTIVITY DATA
A. Mild oxidizing solution capable of reacting with reducing materials.
B. Contact with metals should be avoided. Transition metal ion contamination promotes decomposition.
C. Best stored in inert polyethylene, polypropylene, or Teflon containers.

MSDS 111
LCP 1846

VIII. SPECIAL PRECAUTIONS

A. Protective Measures:

1. Acid-rated gloves and safety glasses should be worn when handling or transferring this solution.
2. Avoid contact with eyes, skin, and clothing.
3. Wash hands (contacted areas) thoroughly after handling.
4. Spills should be diluted with water and flushed to a sanitary sewer system.

IX. ENVIRONMENTAL PROTECTION

A. The considerable solubility of the dissolved salts provides adequate protection against buildup of crystals. The diluted aqueous solution provides nonhazardous waste. Can be burned in an incinerator when diluted.

"To the best of our knowledge the information contained herein is correct. All chemicals may present unknown health hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards which exist. Final determination of suitability of the chemical is the sole responsibility of the user. Users of any chemical should satisfy themselves that the conditions and methods of use assure that the chemical is used safely.

NO REPRESENTATIONS OR WARRANTIES, EITHER EXPRESSED OR IMPLIED, OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE OR ANY OTHER NATURE ARE MADE HEREUNDER WITH RESPECT TO THE INFORMATION CONTAINED HEREIN OR THE CHEMICAL TO WHICH THE INFORMATION REFERS."

THIS MATERIAL IS EXPERIMENTAL AND ANY HAZARDS ASSOCIATED WITH IT HAVE NOT BEEN FULLY CHARACTERIZED. THIS MATERIAL HAS BEEN PROVIDED EXCLUSIVELY FOR TESTING AND EVALUATION IN A LABORATORY ENVIRONMENT BY KNOWLEDGEABLE RESEARCH PERSONNEL.

NCTR

PROTOCOL ADDENDUM FORM

EXPERIMENT NO: E6665.01 DATE: March 23, 1990

EXPERIMENT TITLE : Dermal Toxicity Range-Finding Study Using LP1846 Gun Propellant on Male Miniature Hanford Pigs

CHANGE TO BE DOCUMENTED:

AMENDMENT [X] CLARIFICATION [X] DEVIATION [ ]

CHANGE NUMBER: 1

DATE OR PERIOD OF CHANGE: March 23, 1990

STATEMENT OF CHANGE: See redlined and strikeout portions of pages 3,4 and 6 of protocol

1) Page 3- Change in feed quantity;
2) Page 4- a) Sponsor review prior to setting MTD.;
   b) Change in clinical observations (Number of days and frequency per day); and,
   c) Added day 8 body weight.
3) Page 6- Corrected time frame.

REASON FOR CHANGE:

Clarification of procedures.

STUDY DIRECTOR: William M. Witt DATE: March 23, 1990
William M. Witt, DVM, Ph.D.

SPONSOR NOTIFIED: PHONE [ ] LETTER [X]

SPONSOR NOTIFICATION DATE: March 23, 1990

cc: E6665 files

Josephine Reed	Dr. Winifred Palmer	Dan Brand
Dr. Tom Bucci	Dr. Dale Thurman	Paul Gosnell
Dr. Robert Parker	Kathy Carroll	Bob Harmon
Elijah Smith	Jim Martin	Charlene Kolafa

FILE: C:\WP50\WITT\AMEND1.TXT

Temperature: 23°± 3°C
Relative Humidity: 50 ± 10%
Light/Dark Ratio: 12 hr/12 hr
Clinical Surveillance: Daily
Bedding Changes: Daily around water source; twice-weekly for entire run
Feed: Purina Commercial miniature pig diet
Frequency: once per day (800 - 1200 ~~gram 1 kilogram~~ allotments)
Contaminants in Feed That are Expected to Interfere with Study: None
Water: Filtered
Frequency: ***Ad Libitum***
Contaminants in Water That are Expected to Interfere with Study: None

4.5 ANIMAL MODEL

Species/Strain: Hanford Miniature Pig (Charles Rivers)
Sex: male
Age: 4-5 months old at delivery
Weight: 21-27 Kg at delivery
Procedure for Individual Animal Identification: Supplier identification will be used.

Young adult male Hanford Miniature Swine (Charles Rivers) will be purchased and shipped to NCTR and will be held for a 14-day acclimation period. Physical examinations, base-line hematology/microbiology/clinical chemistry and conditioning to restraint slings will be performed during the acclimation period.

5.0 EXPERIMENT DESIGN

Protocol Duration: 15 Days
Route of Administration: Dermal application (topical application to skin)
Frequency of Administration: Single Dose
Dose Levels (mg LP1846/Kg body weight): To be determined
Number Treated Animals Required/Dose Level: 1 male (4 males total)
Number Control Animals Required: 1 male
Number of Animals Required for Possible Replicate: 5 additional males
Weighing Schedule: Day of treatment, then weekly, and at sacrifice
Biologic Sample Requirements: Blood samples (on day of allocation, day 1 Time 0, 2h, 4h, 8h, and 24h and once daily days 2 through 14 and at necropsy (day 15).

The Rangefinding will use a total of 5 animals per replicate (two replicates may be required). The animals will be randomly assigned, one to each treatment and control. The exposure will consist of a single topical application of neat agent to the skin. The exposed skin will neither be covered, wiped, nor washed once the agent is applied. The exposure area for the control and high dose pigs will encompass approximately 15% of skin area. Skin area to be exposed for each animal shall be calculated using the following formula:

$$\text{Body Surface Area (cm}^2\text{)} = K \times BW^{2/3}$$

Where: K = 9.95 and BW = Body Weight in grams

The upper-middle, low-middle and low dose pigs will receive that amount of neat agent to cover 7.5, 3.8 and 1.9% of the surface area (approximately one-half, one-fourth and one-eight the calculated high dose volume), respectively. All dosing will be accomplished with the animals in a restraint sling (Charles Rivers) as previously described by Panepinto *et al* (1983). The animals will remain in the sling 4 hours post-exposure at which time they will be placed in individual runs. The animals will be returned to the slings only for blood sampling and physical examination during the remainder of the 14 day test period. The ~~highest~~ dose level to be selected for Phase I of the definitive toxicity test will be selected after reviewing the rangefinding results with the sponsor ~~the lowest level that produces Observable Adverse Effects (i.e., lethality, morbidity, excessive skin irritation)~~. This dose level will be termed the Maximum Tolerated Dose (MTD). The blood sampling intervals will be adjusted if necessary to establish the time to maximum methemoglobin level and the time required for recovery to the background methemoglobin level in an effort to accurately follow the kinetics of this process. The animals will be observed twice daily except during study days 2 through 4 where clinical observations will be made ~~at least~~ four (4) times daily ~~for clinical signs of toxicity~~. Skin irritation will be graded according to modification of the method of Draize (1944) (see attached SOP). Body weights will be obtained on the day of exposure, study day 8 and at sacrifice.

5.1 PATHOLOGY

Methemoglobin levels will be determined directly from whole blood immediately after sampling using an IL282 Co-Oximeter (Instrumentation Laboratory Inc., Lexington, MA). Hematology will be done on days 1-5, 8-12 and 15 and will include a complete blood cell count, including cell morphology assessment, hemoglobin concentration, hematocrit, mean corpuscular volume, % red cells with Heinz bodies, and, starting 3 days post-exposure, reticulocyte counts. Clinical chemistry samples to determine the pig liver and kidney function (BUN, total protein, creatinine, albumin and ASAT) will be collected on days 1 - 4, 8 and 15. Gross necropsies will be performed on all moribound animals and animals which die on test. At the termination of the study (day 15), all surviving animals will be euthanized by *iv* injection of T-61 Euthanasia Solution (0.3 ml/Kg) and necropsied. Histopathology will be performed on sections of skin exposed to the test and control articles (both representative samples and lesions occurring in these areas), as well as on any gross lesions observed during the necropsies. An additional skin tissue sample will be collected from a site that is approximately 5.0 cm away from the edge of the treatment area.

5.2 DATA COLLECTION/RECORDS TO BE MAINTAINED

Data collection (body weights, clinical observations, mortality data and day (date) of

9.0 CARE AND USE OF LABORATORY ANIMALS

This protocol will be conducted in accordance to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 85-23, Revised 1985). The NCTR Animal Care and Use Committee will monitor animal care issues of this protocol.

10.0 SIGNIFICANCE AND BENEFITS

LP1846 liquid gun propellant is under development as a possible substitute for current solid gun propellants. Limited toxicity data are available on several liquid gun propellant formulations as well as their major components. Data confirms that they are toxic in rabbits, guinea pigs and miniature pigs. The dermal toxicity assay is an ***in vivo*** test that would provide toxicologic information about the risk from dermal absorption of LP1846.

11.0 TIME FRAME

Study #	Allocation ~~Select~~	Start	Necropsy ~~End Study~~
E6665.01A	22-23-FEB-90 ~~TBA~~	26-FEB-90 ~~TBA~~	12-MAR-90 ~~TBA~~
E6665.01B	16-MAR-90	19-MAR-90	02-APR-90

12.0 REFERENCES

1. Freedman, Eli, ed. An Outline of Liquid Gun Propellants, USAARD, Aberdeen Proving Ground, MD.

2. Draize, J.H. *et al.* Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther., 82:377-390 (1944).

3. Panepinto, L.M. *et al.* A comfortable, minimum stress method of restraint for Yucatan miniature swine. Lab. Anim. Sci., 33:95-97 (1983).

NCTR

PROTOCOL ADDENDUM FORM

EXPERIMENT NO: E6665.01 DATE: March 23, 1990

EXPERIMENT TITLE : Dermal Toxicity Range-Finding Study Using LP1846 Gun Propellant on Male Miniature Hanford Pigs

CHANGE TO BE DOCUMENTED:

AMENDMENT [X] CLARIFICATION [ ] DEVIATION [ ]

CHANGE NUMBER: 2

DATE OR PERIOD OF CHANGE: March 23, 1990

STATEMENT OF CHANGE: Appending Sponsor signature to Protocol

REASON FOR CHANGE:

Sponsor approved protocol prior to initiation of the study per telephone conversation. Signature sheet had been sent to her in advance of the study. Sponsors signature was dated 2/9/90, which was also prior to start of study.

STUDY DIRECTOR: William M. Witt DATE: March 23, 1990
William M. Witt, DVM, Ph.D.

SPONSOR NOTIFIED: PHONE [X] LETTER []

SPONSOR NOTIFICATION DATE: February 9, 1990

cc: E6665 files

Josephine Reed	Dr. Winifred Palmer	Dan Brand
Dr. Tom Bucci	Dr. Dale Thurman	Paul Gosnell
Dr. Robert Parker	Kathy Carroll	Bob Harmon
Elijah Smith	Jim Martin	Charlene Kolafa

FILE: C:\WP50\WITT\AMEND2.TXT

GLP: RAW DATA
Human Subject: ______ Yes XX No
Controlled Sub.: ______ Yes XX No

EXP. NO. E6665.01

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
SHORT-TERM PROTOCOL

TITLE: Dermal Toxicity Range-Finding Study Using LP1846 Gun Propellant on Male Miniature Hanford Pigs

PROGRAM AREA: DOD IAG #224-89-0005

William M. Witt — DATE: 26 Jan 90
Principal Investigator / Division Director

for Dr Dale Thurman — DATE: 26 Jan 90
Co-Principal Investigator

DATE: 1/26/90
Co-Principal Investigator

DATE: Jan 26, 1990
Co-Principal Investigator

for Fred Kadlubar — DATE: 2/2/90
Assoc. Dir. for Research

DATE:
Assoc. Dir. for Research Services

DATE:
Assoc. Dir. for Management

DATE:
ACUC Chairman

DATE:
Safety Officer

Approved By: — DATE: 2/9/90
Contracting Officer's Representative, USABRDL

Approved By: — DATE:
Director, NCTR

ENCL

DEPARTMENT OF HEALTH & HUMAN SERVICES

Memorandum

Date March 23, 1990

From William M. Witt (HFT-240)

Subject Protocol 6665.01

To Chairman, Animal Care and Use Committee (HFT-140)

I wish to request a revision in Part I. B. Technical Service Needs of the ACUC-approved LACUF for Protocol 6665.01. The change is as follows: The daily moribund/death check and frequency is to be changed from "4 times per day for first 4 days of exposure, then 2 times per day to study end" to "twice daily except during study days 2 through 4 where clinical observations will be made four times daily." This change is requested because dosing does not occur until approximately 11:30 a.m., which would necessitate three clinical observations being made and entered into the INLIFE System within five hours. We have found that this frequency on the day of dosing is not necessary.

William M. Witt

William M. Witt, (HFT-240)

NCTR

PROTOCOL ADDENDUM FORM

EXPERIMENT NO: E6665.01 DATE: March 23, 1990

EXPERIMENT TITLE : Dermal Toxicity Range-Finding Study Using LP1846 Gun Propellant on Male Miniature Hanford Pigs

CHANGE TO BE DOCUMENTED:

AMENDMENT [X] CLARIFICATION [ ] DEVIATION [ ]

CHANGE NUMBER: 3

DATE OR PERIOD OF CHANGE: March 28, 1990

STATEMENT OF CHANGE: Change in Percent Surface Area treated for Replicate B

REASON FOR CHANGE: Fine pointing test range of Replicate B to have 15, 12.5, 10, and 5 percent of the surface area treated. These areas were selected after data review and consultation with sponsor.

STUDY DIRECTOR: William M. Witt DATE: March 28, 1990
William M. Witt, DVM, Ph.D.

SPONSOR NOTIFIED: PHONE [X] LETTER [X]

SPONSOR NOTIFICATION DATE: March 28, 1990

cc: E6665 files ✓

Josephine Reed	Dr. Winifred Palmer	Dan Brand
Dr. Tom Bucci	Dr. Dale Thurman	Paul Gosnell
Dr. Robert Parker	Kathy Carroll	Bob Harmon
Elijah Smith	Jim Martin	Charlene Kolafa

FILE: C:\WP51\WITT\AMEND3.TXT

NCTR

PROTOCOL ADDENDUM FORM

EXPERIMENT NO: E6665.01 DATE: March 23, 1990

EXPERIMENT TITLE : Dermal Toxicity Range-Finding Study Using LP1846 Gun Propellant on Male Miniature Hanford Pigs

CHANGE TO BE DOCUMENTED:

AMENDMENT [ ] CLARIFICATION [X] DEVIATION [ ]

CHANGE NUMBER: 4

DATE OR PERIOD OF CHANGE: March 28, 1990

STATEMENT OF CHANGE: Clarification of change in feed quantity in Protocol Addendum Form Change Number 1 Dated March 23, 1990. Feed Quantity Weights should have been noted as kilograms not grams (0.8 - 1.2 Kilograms)[page 3 of protocol].

REASON FOR CHANGE: Maintain consistancy in feeder weight units.

STUDY DIRECTOR: William M. Witt DATE: March 28, 1990
William M. Witt, DVM, Ph.D.

SPONSOR NOTIFIED: PHONE [] LETTER [X]

SPONSOR NOTIFICATION DATE: March 28, 1990

cc: E6665 files

Josephine Reed	Dr. Winifred Palmer	Dan Brand
Dr. Tom Bucci	Dr. Dale Thurman	Paul Gosnell
Dr. Robert Parker	Kathy Carroll	Bob Harmon
Elijah Smith	Jim Martin	Charlene Kolafa

FILE: C:\WP51\WITT\AMEND4.TXT

Temperature: 23°± 3°C
Relative Humidity: 50 ± 10%
Light/Dark Ratio: 12 hr/12 hr
Clinical Surveillance: Daily
Bedding Changes: Daily around water source; twice-weekly for entire run
Feed: Purina Commercial miniature pig diet
Frequency: once per day (0.8 - 1.2 Kilogram~~-1 kilogram~~ allotments)
Contaminants in Feed That are Expected to Interfere with Study: None
Water: Filtered
Frequency: *Ad Libitum*
Contaminants in Water That are Expected to Interfere with Study: None

4.5 ANIMAL MODEL

Species/Strain: Hanford Miniature Pig (Charles Rivers)
Sex: male
Age: 4-5 months old at delivery
Weight: 21-27 Kg at delivery
Procedure for Individual Animal Identification: Supplier identification will be used.

Young adult male Hanford Miniature Swine (Charles Rivers) will be purchased and shipped to NCTR and will be held for a 14-day acclimation period. Physical examinations, base-line hematology/microbiology/clinical chemistry and conditioning to restraint slings will be performed during the acclimation period.

5.0 EXPERIMENT DESIGN

Protocol Duration: 15 Days
Route of Administration: Dermal application (topical application to skin)
Frequency of Administration: Single Dose
Dose Levels (mg LP1846/Kg body weight): To be determined
Number Treated Animals Required/Dose Level: 1 male (4 males total)
Number Control Animals Required: 1 male
Number of Animals Required for Possible Replicate: 5 additional males
Weighing Schedule: Day of treatment, then weekly, and at sacrifice
Biologic Sample Requirements: Blood samples (on day of allocation, day 1 Time 0, 2h, 4h, 8h, and 24h and once daily days 2 through 14 and at necropsy (day 15).

The Rangefinding will use a total of 5 animals per replicate (two replicates may be required). The animals will be randomly assigned, one to each treatment and control. The exposure will consist of a single topical application of neat agent to the skin. The exposed skin will neither be covered, wiped, nor washed once the agent is applied. The exposure area for the control and high dose pigs will encompass approximately 15% of skin area. Skin area to be exposed for each animal shall be calculated using the following formula:

NCTR

PROTOCOL ADDENDUM FORM

EXPERIMENT NO: E6665.01 DATE: SEPTEMBER 10, 1990

EXPERIMENT TITLE: Dermal Toxicity Range-Finding Study Using LP1846 Gun Propellant on Male Miniature Hanford Pigs

CHANGE TO BE DOCUMENTED:

AMENDMENT [X] CLARIFICATION [X] DEVIATION [X]

CHANGE NUMBER: 5

DATE OR PERIOD OF CHANGE: SEPTEMBER 10, 1990

STATEMENT OF CHANGE:

I. Deviation in protocol.

4.5 ANIMAL MODEL - amended to read:

Species/Strain: Hanford Miniature Pig (Charles River Laboratories, Inc. [CRL])
Sex: Male
Age: 4-7 months old (+/- 0.5 mo) at shipment
Weight: 20-24 kg (+/- 2 kg) at shipment
Procedure for Individual Animal Identification: Supplier identification will be used.

Young adult male Hanford Miniature Swine (CRL) will be purchased and shipped to NCTR and will be held for a minimum of 14 days acclimation. Physical examinations, base-line hematology/microbiology/clinical chemistry and conditioning to restraint slings will be performed during the acclimation period.

REASON FOR CHANGE:

Deviation of original protocol due to supplier's (CRL) inability to meet the original age/weight specifications for the number of animals and specific delivery dates required for the protocol.

II. Clarification of procedure.

After discussion with Sponsor following completion of Replicate A, method to be used for applying LP1846 to the skin surface was as follows:

The calculated volume of neat LP1846 was dispensed into a clean beaker via the Digiflex Automated Dispensing Syringe. A 4"X 4" gauze sponge was saturated with the agent and the sponge used to apply the material to the skin with gentle rubbing motion. The procedure was repeated (wringing the sponge out frequently) until all the designated volume of agent was applied to the skin.

STUDY DIRECTOR: William M. Witt
William M. Witt, D.V.M., PH.D. DATE: SEPTEMBER 10, 1990

SPONSOR NOTIFIED: PHONE [ ] LETTER [X]

SPONSOR NOTIFICATION DATE: SEPTEMBER 10, 1990

cc: E6665 files

Josephine Reed	Dr. Winifred Palmer	Dan Brand
Dr. Robert Parker	Dr. Dale Thurman	Paul Gosnell
Elijah Smith	Bob Harmon	Kathy Carroll
Charlene Kolafa	Jim Martin	NCTR Safety Office

A P P E N D I X B

D R A F T A N A L Y T I C A L P R O C E D U R E S

APPROVED:

FAX HEADER SHEET

TO: B.H Witt

ATTENTION: NCTR

FAX: 1-501-541-4030

FROM: Jody Wojciechowski

ADDRESS: USA BALLISTIC RESEARCH LABORATORY
SLCBR-IB-B
ABERDEEN PROVING GROUND, MD 21005-5066

PHONE: 301-278-6160

FAX: 301-278-6159

NO. OF PAGES (INCLUDING HEADER): 4

COMMENTS: This is a draft as of 15 June 89. When the final version is completed, I will forward.

DRAFT OF ANALITICAL PROCEDURES, 15 JUNE 1989, R. SASSE'

TABLE II. EXCESS HNO3 IN LP 1846-06 LOT 06

WEIGHT %	SAMPLE MASS g
0.0030	35.8778
0.0016	35.9070
0.0024	35.8933
0.0014	35.9157
0.0042	35.9635

MEAN VALUE, wt.%: 0.00252

STANDARD DEVIATION*:	(+/-)
Unbiased	0.00289
Biased	0.00113

*Quoted precisions, shown in TABLES I and II, do not include the estimate for the precision of the standard base, which in this example was 0.2730 (+/-) 0.0021 M.

Although I have not yet run Karl Fisher, comments were offered by M. Decker: " Water content of the HAN, TEAN or the propellant are determined using the standard Karl Fisher titrants or the proprietary reagent, 'Hydranal'. The unknown solution must be acidified to prevent amines from reacting with the titrant. A mixture of 90% methanol and 10% glacial acetic acid prevent the amine reaction."

Also, total nitrate concentration is measured by ultraviolet spectrophotometry at 302 nm using 1.0 cm cells. The extinction coefficient was measured to be 7.225 l/M-cm by Richard Biddle in 1985, of Morton Thiokol, over the concentration range to 0.13 M. In the narrow concentration range of 0.11 to 0.13 M, Biddle also reported an extinction coefficient of 7.163 l/M-cm. In neither case were individual values given nor were deviations reported. Thus, it is possible that the absorbence of nitrate is not a linear function of concentration and this is consistent with the density/concentration relationships developed previously by Sasse'.

Some FTIR results have been published; however, analytical methods have yet to be developed.

DRAFT OF ANALITICAL PROCEDURES, 15 JUNE 1989, R. SASSE'

ANALYSIS OF EXCESS STRONG ACID IN LP

Strong acid, if present in moderate amounts in LP, can be titrated directly with strong aqueous base. The resulting typical "S" shaped curve can be interpreted routinely. Such is the case for solutions more acidic than about 0.20 weight percent. At lesser acid concentrations the titration curve is not developed. Titrating pure HAN represents the titration of a buffered common-ion situation having no equivalence end point. To analyze samples containing a small amount of excess acid a spiking technique was adopted where 1 to 4ml of nitric acid was added which allowed the development of an "S" shaped curve. This method provided the added benefit of detecting any degree of acid deficiency. Typical titration graphs are shown in Figure 1 for the titration of pure HAN and also a HAN sample spiked with 4.00 mL of 0.1M HNO3. The procedure is as follows:

> Samples are prepared by weighing 28 grams of LP and diluting with 40 mL of distilled water. Samples are spiked with 1 to 4 mL of 0.25 to 0.30 M HNO3 and titrated with 0.2 to 0.3 M NaOH.

Typical analysis for propellant lot 1846-06, having a density of 1.44519 (+/-) 0.00014 at 20.0 C, is given in Table 1. Deviations reflect the precision of the titration, using a bias estimate for error where sample population was taken at N rather than N-1. Such values are given in tables 1 and 2 which also show biased standard deaviations for comparison. No protocol has been established but it is suggested that a minimum of five samples be analyzed for qualification requirements.

TABLE 1. HAN AND TEAN ANALYSIS OF LP 1846-06 LOT 06

SAMPLE MASS g	HAN %	TEAN %	HAN/TEAN ratio
0.2994	61.2150	20.0451	3.0539
0.3246	61.2755	19.9200	3.0761
0.3064	61.1151	20.1117	3.0388
0.3116	61.3617	19.9005	3.0834
0.3131	61.2155	19.8350	3.0862
0.3223	61.1544	19.6815	3.1072
0.3221	61.2751	20.0476	3.0565
0.3404	61.2039	19.9052	3.0748
MEAN VALUES, wt %:	61.2270	19.9308	3.0721
STANDARD DEVIATIONS:	(+/-)	(+/-)	(+/-)
Unbiased	0.0769	0.1376	0.0216
Biased	0.0719	0.1287	0.0202

DRAFT OF ANALITICAL PROCEDURES, 15 JUNE 1989, R. SASSE'

ANALYSIS OF HYDROXYLAMMONIUM NITRATE BASED LIQUID PROPELLANTS

Liquid propellants (LP) have been, and continue to be, a subject of active study at the Ballistics Research and other Laboratories for a number of years. Two candidate systems chosen for extensive evaluation are identified as 1845 and 1846 - both of which are homogeneous mixtures of hydroxylammonium nitrate (HAN), triethanolammonium nitrate (TEAN) and water. The propellants are formulated to be stoichiometric with respect to the combustion products, carbon dioxide, nitrogen and water. Ideal or target compositions are given in Table I.

TABLE I. CHEMICAL COMPOSITION OF LIQUID PROPELLANT

PROPELLANT COMPOSITION

TYPE	HAN		TEAN		WATER	
	wt. %	M	wt. %	M	wt. %	M
1845	63.23	9.62	19.96	1.38	16.81	13.64
1846	60.79	9.09	19.19	1.30	20.02	15.93

Acceptable deviations from target concentrations have been set at (+/-) 0.5 weight percent with respect to HAN, TEAN and water concentrations. These are the current limits tolerated in purchasing and contracting actions. Furthermore, an excess nitric acid limit in HAN is being considered, at about 0.1 weight percent, as well as a lower boundary representing some limit on the degree of acid deficiency allowed. Clearly, we have to become more precise and perhaps include a lower limit for ammonium ion concentration.

In recent times, analytical reports have been published by the same author where conflicting statements have been presented among a collection of reports, or various authors have recommended different approaches. This situation reflects progress as opposed to controversy; however, it seems prudent at this time to identify the analytical methods currently used by BRL.

Early attempts to titrate LP with aqueous base gave but one end point near a pH of 10 for the combined HAN and TEAN concentrations. This is the direct result of the respective pK's being too close in value where the equivalence points for titrating HAN and TEAN indivally are at pH's of 8.8 and 9.7 respectfully. This situation was avoided by adding a small amount of acetone that quantitatively reacts with HAN to form an oxime and nitric acid. Then nitric acid and TEAN could be titrated with base, yielding two distinct end points, one at pH of 5 and the other at a pH of 10. The procedure is as follows:

> Samples of 0.25 to 0.30 grams of LP are diluted with 50 mL of distilled water to which 2.0 mL of acetone are added. Titrations are performed with 0.25 to 0.30 M of NaOH.

A P P E N D I X C

R A T S K I N S U R F A C E

A R E A C O V E R A G E B Y L P 1 8 4 6

Date: August 1, 1990

To: Dr. William Witt
Principal Investigator
Veterinary Services

From: Paul Gosnell
Co-principal Investigator
Pathology Services

Subject: Determination of LP1846 Volume to Cover Required Skin Surface Area.

Prior to the start of the range-finding LP1846 was tested for its wetting capabilities on rat skin. The procedure followed was the same as with the glycerol on human skin. The rat skin was collected and used while fresh. Application of 1ml of LP1846 wetted approx. 50cm^2. This value was used during the range-finding and continues to be used during the present study.

A P P E N D I X D

V I T A M I N C A N A L Y S I S O F B E D D I N G

04/02/90 NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH PAGE 1
DIVISIONS OF CHEMISTRY AND MICROBIOLOGY

DETAILED SURVEILLANCE REPORT
EXPERIMENT 0022

4-2-90
KM

SAMPLE NO/ SAMPLE TYPE	SAMPLE ID	LABORATORY/ TEST/SPECIMEN	RESULTS/OBSERVATIONS	COMMENTS/RECOMMENDATION
900316-030	DATE RECD: 6-FEB-1990	Chemistry		
CUST#: 00220400137	DATE COLL: 6-FEB-1990	VITAMIN E	< MDL #	VITAMIN C WAS NOT PRESENT
LOT: 100000222662015	BLDG/RM: 4/100			
EXPT: 0022				
TYPE: BEDDING				
SUBTYPE: SHIPMENT				

APR 3 3 :C PI '90
RECD-DAH

APPENDIX E

DEVIATIONS OF ANIMAL CARE

DOCUMENTATION

EXPERIMENT: 6665.01 DATE: 3/29/90

BUILDING: 14 ROOM: 101

InLife printout for container weights collected March 7, 1990 on animal #1 showed the new container weight was less than the old container weight. The old container weight was 1515.1 gms., and the new container weight was 900.2 gms. Animal #1 received the correct amount of feed (900.2 gms.) but the technician failed to weigh the container along with the new feed.

Elijah Smith
SIGNATURE

3-29-90
DATE

To be filled in by Principal Investigator. (Please check one, and/or comment, as applies.)

This deviation should _____ should not X effect the outcome of this study.

Comments: _____

_____

cc: Dr. Witt
Dr. Robert Parker
Mr. Mark Moore
Mr. Bob Harmon
QA
Computer room
Mr. Elijah Smith

William M. Witt
Principal Investigator's Signature

Check one:

Approved: _____ Date: _____

Disapproved: _____ Date: _____

Acknowledged: _____ Date: _____

DOCUMENTATION

EXPERIMENT: 6665.01 DATE: 3/28/90

BUILDING: 14 ROOM: 101

InLife transaction report for clinical observation Expt. 6665.01 on Wednesday, 2/28/90 thru Friday, 3/2/90 shows that clinical observations were entered three times each day, rather than four times daily as required by experimental protocol.

Elijah Smith
SIGNATURE

3-29-90
DATE

To be filled in by Principal Investigator. (Please check one, and/or comment, as applies.)

This deviation should _____ should not X effect the outcome of this study.

Comments: _____

_____

cc: Dr. Witt
Dr. Robert Parker
Mr. Mark Moore
Mr. Bob Harmon
QA
Computer Room
Mr. Elijah Smith

William M. Witt
Principal Investigator's Signature

Check one:
Approved: _____ Date: _____
Disapproved: _____ Date: _____
Acknowledged: _____ Date: _____

DOCUMENTATION

EXPERIMENT: 6665.01 DATE: 3/28/90

BUILDING: 14 ROOM: 101

InLife transaction reports for Animals 1-5 (Inclusive) on Exp. 6665.01 on 3/3, 3/4, 3/5 and 3/6 reveals that clinical observation were performed once daily, rather than twice daily as required by experimental protocol.

Elijah Smith
SIGNATURE

3-29-90
DATE

To be filled in by Principal Investigator. (Please check one, and/or comment, as applies.)

This deviation should _____ should not X effect the outcome of this study.

Comments: _____

_____

cc: Dr. Witt
Dr. Robert Parker
Mr. Mark Moore
Mr. Bob Harmon
QA
Computer Room
Mr. Elijah Smith

William R. Witt
Principal Investigator's Signature

Check one:
Approved: _____ Date: _____
Disapproved: _____ Date: _____
Acknowledged: _____ Date: _____

APPENDIX F

LIST OF ABBREVIATIONS

LIST OF ABBREVIATIONS

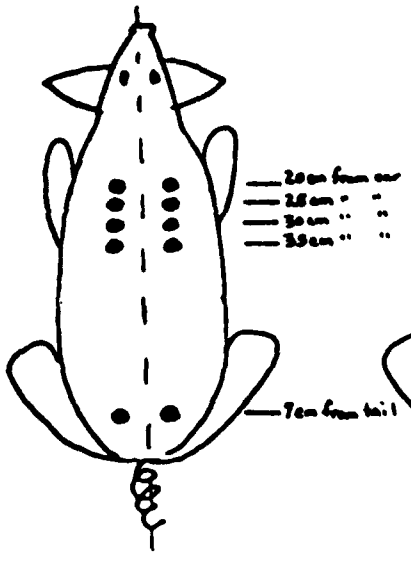
ALB	albumin
AST	aspartate aminotransferase
BUN	blood urea nitrogen
BW	body weight
C	centigrade
cc_2	cubic centimeters
cm^2	square centimeters
cp	centipoise
CREA	creatinine
EOS_2	eosinophils
Fe^{+2}	ferrous iron
Fe^{+3}	ferric iron
fl	femtoliter
g	grams
g/dl	grams per deciliter
HAN	hydroxylammonium nitrate
Hct	hematocrit
Hgb	hemoglobin
hr	hours
iv	intravenous
K	formula constant (9.95 in this case)
LYM	lymphocytes
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin content
MCV	mean corpuscular volume
mg/dl	milligram per deciliter
ml	milliliter
ml/kg	milliliter per kilogram
mm	millimeter
MON	monoctyes
MTD	maximum tolerated dose
NCTR	National Center for Toxicological Research
PCI	primary cutaneous irritation index
pg	picogram
RBC	red blood cell (erythrocyte)
RETIC	reticuloctyes
SEG	segmented white blood cell (neutrophils)
SOPs	standard operation procedures
SSAE/SSAEs	skin surface area exposure(s)
TEAN	triethanolammonium nitrate
TP	total protein
U/L	international unit per liter
U.S. Army	United States Army
WBC	white blood cells

APPENDIX G

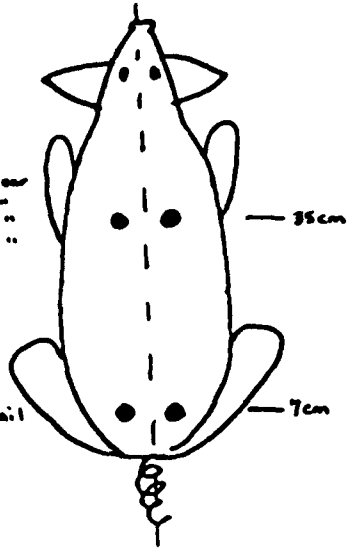
SKIN SAMPLE SITES FOR HISTOPATHOLOGY

SKIN SAMPLE SITES FOR HISTOPATHOLOGY

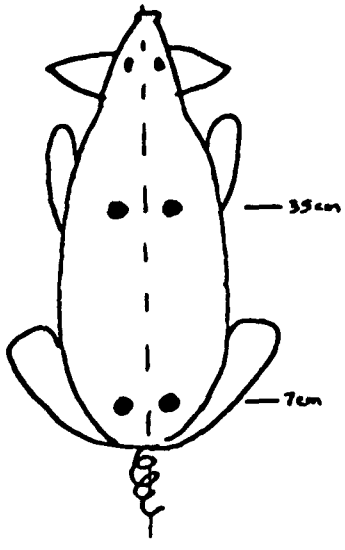
Replicate A



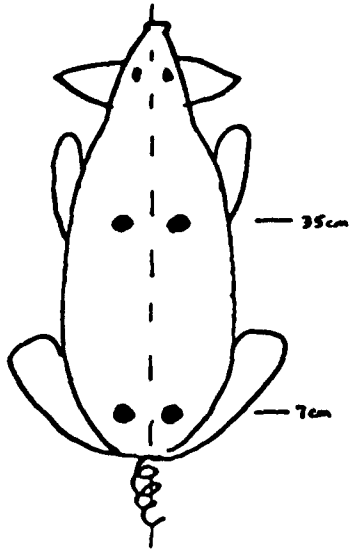
#118-8
15% LP1846



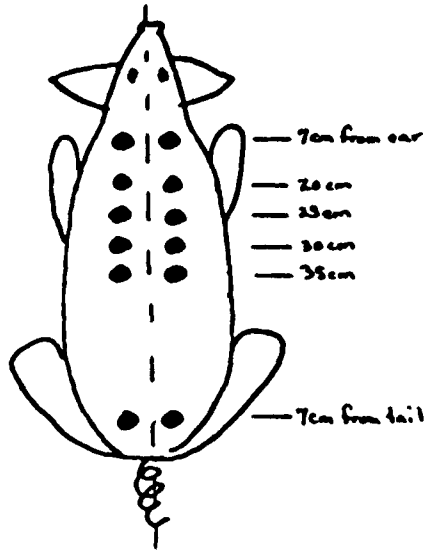
#95-2
7.5% LP1846



#83-6
3.8% LP1846

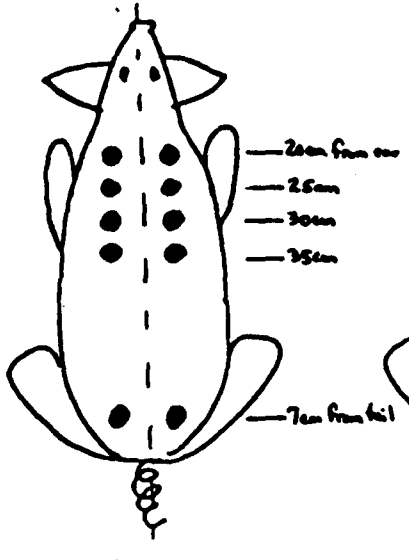


#99-10
1.9% LP1846

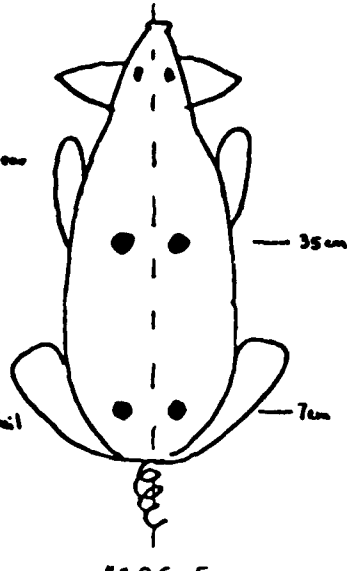


#109-4
Control

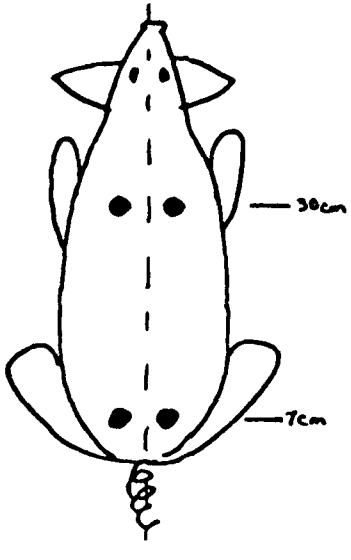
Replicate B



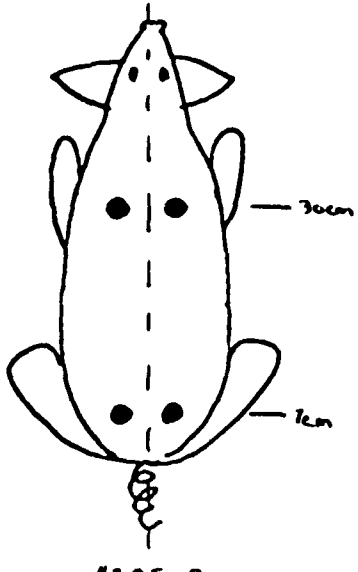
#84-4
15% LP1846



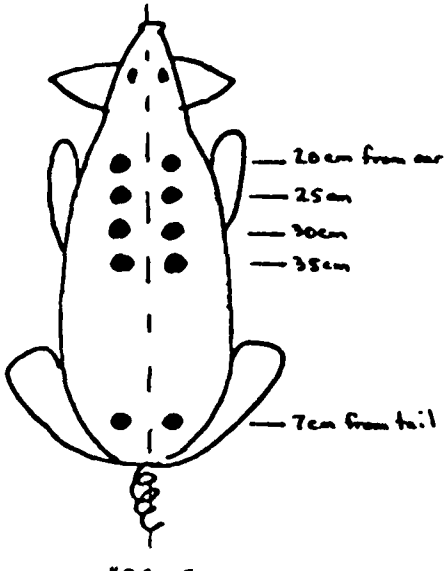
#106-5
12.5% LP1846



#104-6
10% LP1846



#105-8
5% LP1846



#91-6
Control

APPENDIX H

HISTOPATHOLOGY GRADING OF SKIN SAMPLES

HISTOPATHOLOGY GRADING OF SKIN SAMPLES

Replicate A				Replicate B			
Animal	Smpl	Score	Location	Animal	Smpl	Score	Location
#118-8	A	3	20cm from ear (L)	# 84-4	A	2	20cm from ear (L)
	B	3	20cm from ear (R)		B	2	20cm from ear (R)
	C	3	25cm from ear (L)		C	3	25cm from ear (L)
	D	3	25cm from ear (R)		D	4	25cm from ear (R)
	E	3	30cm from ear (L)		E	4	30cm from ear (L)
	F	3	30cm from ear (R)		F	4+	30cm from ear (R)
	G	2	35cm from ear (L)		G	4	35cm from ear (L)
	H	2	35cm from ear (R)		H	4+	35cm from ear (R)
	I	2	7cm from tail (L)		I	1	7cm from tail (L)
	J	2	7cm from tail (R)		J	1	7cm from tail (R)
# 95-2	A	4-	35cm from ear (L)	#106-5	A	5	35cm from ear (L)
	B	3	35cm from ear (R)		B	5	35cm from ear (R)
	C	0	7cm from tail (L)		C	2	7cm from tail (L)
	D	0	7cm from tail (R)		D	2	7cm from tail (R)
# 83-6	A	3+	35cm from ear (L)	#104-6	A	3	35cm from ear (L)
	B	4-	35cm from ear (R)		B	3	35cm from ear (R)
	C	1	7cm from tail (L)		C	1	7cm from tail (L)
	D	1	7cm from tail (R)		D	1	7cm from tail (R)
# 99-10	A	4	35cm from ear (L)	#105-8	A	3	35cm from ear (L)
	B	4	35cm from ear (R)		B	3	35cm from ear (R)
	C	1	7cm from tail (L)		C	3	7cm from tail (L)
	D	1	7cm from tail (R)		D	3	7cm from tail (R)
#109-4	A	1	7cm from ear (L)	# 91-6	A	1	20cm from ear (L)
	B	2	7cm from ear (R)		B	1	20cm from ear (R)
	C	1	20cm from ear (L)		C	1	25cm from ear (L)
	D	1	20cm from ear (R)		D	1	25cm from ear (R)
	E	2	25cm from ear (L)		E	1	30cm from ear (L)
	F	2	25cm from ear (R)		F	1	30cm from ear (R)
	G	1	30cm from ear (L)		G	1	35cm from ear (L)
	H	1	30cm from ear (R)		H	1	35cm from ear (R)
	I	1	35cm from ear (L)		I	1	7cm from tail (L)
	J	1	35cm from ear (R)		J	1	7cm from tail (R)
	K	1	7cm from tail (L)				
	L	2	7cm from tail (R)				

Grading Criteria

Score	Description
0	Very rarely inflammatory cells* present and when present they are widely distributed
1	Very few inflammatory cells (background at most); no edema or other associated inflammatory changes

2 Slight increase in inflammatory cells; can have very mild multifocal edema

3 Moderate numbers of inflammatory cells, multifocal mild edema may (often is) be present

4 Edema is prominent component; often is zonal usually subepidermal; edema is most often present as diffuse distribution but occasionally multifocal

5 Subepidermal, with occasional periadnexal, distribution of edema; increased inflammatory cells up to "marked" level; some focal necrosis of epidermis is present

All changes (unless otherwise stated) are in the papillary layer of the dermis. The epithelium is intact in all tissue sections. A superficial layer of serum exudate is often present on the epidermis in grades 3-5.

* inflammatory cells are nearly always lymphocytes or macrophages; rarely, granulocytes are present

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